ARCHIVES OF PATHOLOGY

VOLUME 20 JULY 1935 NUMBER 1

EARLY INCIDENCE OF SPONTANEOUS MEDIAL DEGENERATION ("ARTERIOSCLEROSIS") IN THE AORTA OF THE RABBIT

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In the course of an attempt to reproduce the transmission of viosterol-induced arteriosclerosis from mother to young as described by Junck,[1] the opportunity presented itself to study the aortas from 125 young rabbits. These rabbits, with few exceptions, were the successive litters of several mothers by a single father. Coming from known stock and being raised under standard conditions, they formed unusually well controlled material for study. The remarkably high incidence of degenerative changes in the media of the aortas of these rabbits forms the basis for this report.

METHOD

The parent rabbits, 6 does and 1 buck, were large, young adult chinchillas.[2] Two females received subcutaneously 0.18 cc. of 1,500 D viosterol [2a] (30,000 rat units), divided into daily doses over a period of one week; 2 received 1.2 cc. (200,000 rat units) in similar fashion. The remaining 2 females served as controls; they as well as the male received no viosterol. At periods varying from one month before to eight months after the administration of the viosterol, the animals were bred. The control mothers were bred at the same time, and also again nearly a year after the beginning of the work. The animals were housed in large breeding cages in a sunny indoor environment. The young were weaned at 6 weeks of age and placed on the standard stock diet fed the parents.[3] The successful breeding of the adults and the satisfactory growth and excellent nutritional state of the young indicated that the diet was a liberal one. The incidence of coccidiosis was sharply limited by the exclusion of fresh vegetables.

This investigation was aided by a grant from the Josiah Macy Jr. Foundation.

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1. Junck, A.: Virchows Arch. f. path. Anat. **283**:265, 1932.

2. The parent rabbits were of a strain used for breeding purposes at Rockland Farms, New City, N. Y.

2a. Viosterol was supplied as 1,500 D vigantol by the Winthrop Chemical Co., New York.

3. The stock diet for rabbits was composed as follows: oats, 50 per cent; rolled oats, 10 per cent; whole wheat, 10 per cent; barley, 10 per cent; bran, 5 per cent; corn, 5 per cent; linseed oil meal, 5 per cent, and molasses-alfalfa (equal parts), 5 per cent. Timothy and alfalfa hay were given ad libitum.

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At ages varying from 1 day to 8 months the rabbits were killed and autopsies performed. In the examination of the younger animals the entire aorta was sectioned serially; in that of the older ones, suitable blocks were removed from the aorta and similarly sectioned.

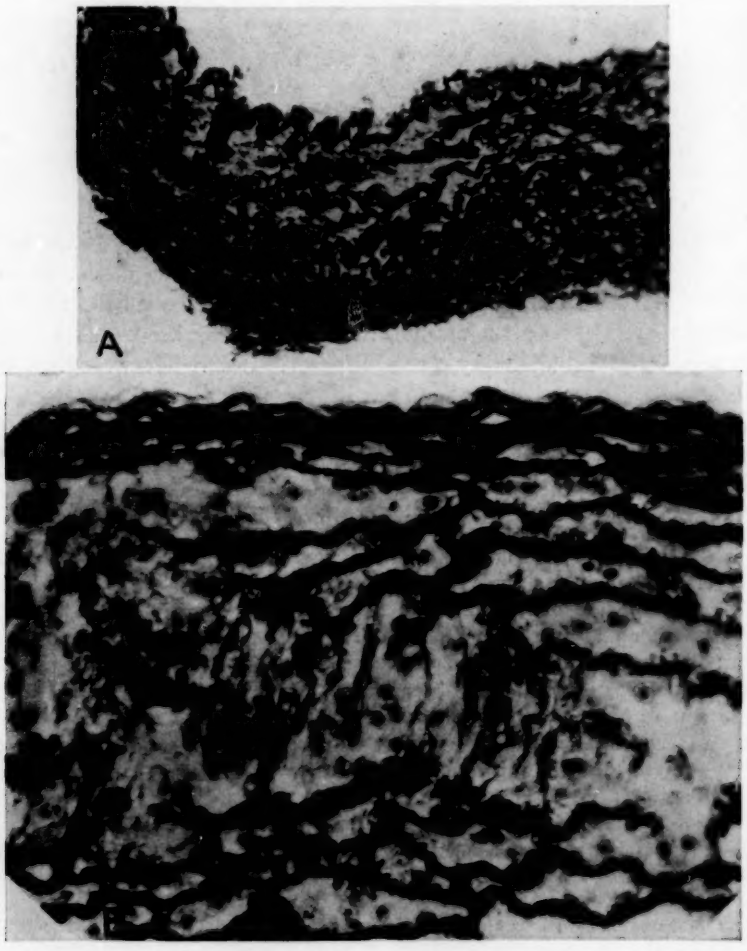

Fig. 1.—*A*, separation, fragmentation and fraying of the medial elastic fibers in the aorta of a 10 day old rabbit. Weigert-Van Gieson stain; × 160. *B*, lesion in a 23 day old rabbit. Delicate elastic fibrils run transversely between interrupted elastic fibers. Note the mononuclear infiltration. Weigert-Van Gieson stain; × 450.

RESULTS

In gross character the aortic lesions ranged from a single minute, slightly raised, grayish-white, round spot, usually in the ascending portion of the arch, to numerous discrete or confluent, round or linear,

wrinkied, often umbilicated, gray or occasionally yellowish, partly calcified plaques, varying in size up to 2 mm. in diameter and involving most of the arch.

Histologic study indicated that the earliest change was swelling, fragmentation and fraying of the elastic fibers in the inner media (fig. 1 *A*). This was associated with an increase in interfibrillar fluid.

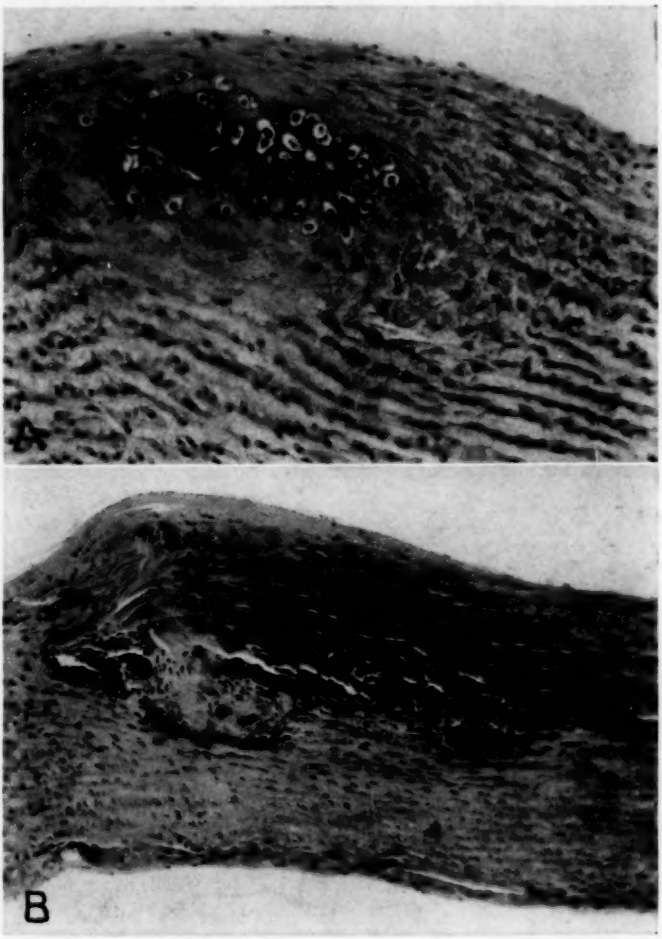

Fig. 2.—*A*, formation of cartilage in a medial aortic lesion of an 8 month old rabbit. Hematoxylin-eosin stain; × 160. *B*, extensive calcification of a lesion at 5 months. Hematoxylin-eosin stain; × 100.

There ensued an infiltration of wandering mononuclear cells between the altered elastic fibers. Often bundles of delicate wavy elastic fibrils ran transversely (radially) between the wandering cells (fig. 1 *B*).

Hyalinization of the interfibrillar ground substance, mucinous degeneration and calcification (fig. 2 *B*) occurred at later stages. Calcium was first laid down along and closely apposed to swollen, taut or fragmented elastic fibers. The presence of fat was not a feature; it was seen only occasionally in late lesions within phagocytes or scattered between the fibers. Small groups of cartilage cells were sometimes observed in the older plaques (fig. 2 *A*). The intima over the lesions was usually raised and later wrinkled. There was a moderate tendency for the endothelial

Occurrence of Aortic Medial Degeneration in Young Rabbits from Mothers Given Viosterol Prior to First Breeding

Mother and Amount of Viosterol in Rat Units	Litter	Period Elapsing Between Administration of Viosterol to Mother and Birth of Litter, Months	Results of Postmortem Examination of Aortas of Young at Ages Given in Months							
			½	1	2	3	4	5	6	8
A	1	0*		—	+ —					—
30,000	2	6	—	+	+ +			+		+ +
	3	9	—	+	—	+ +		+		+
B	1	0	— — — — —	—			+			+
30,000	2	6	—	+	+	+ +		+		+ +
	3	9			+	+ +		+		+
C	1	6	— —	— —	+	+		—		+
200,000	2	9		—	+	+ +		+		+
D	1	2	— —	— —		+		+		+
200,000	2	6	— —		+			+		+ +
	3	9	— — —			+				+
X	1*		— —	—	—			+	—	+
Control	2		— —	—	+	—		—		+ +
	3		—	+	+ +	+		+		+
	4		—	+ +	—	—		—		—
Y	1*		— — —							
Control	2		— — — — +			+		—		—
	3		+	—	+	+		—		+ +
	4			—	+ +	+		—		+
	5			+	+					

* Mother received pregnant; father of litter unknown.
\+ Medial degeneration of aorta present.
— Medial degeneration of aorta absent.

cells to multiply, forming a layer usually one cell thick with nuclei relatively close together. Only rarely was actual thickening of the intima to be observed. Pertinent lesions were not seen in any of the viscera.

The occurrence of aortic lesions in the 125 young rabbits studied is given in the table. The youngest animal in which a lesion was seen was only 10 days old. It was from a mother which did not receive viosterol (fig. 1 *A*). The aorta of another animal, killed at 23 days of age, from another litter of the same mother, also contained a single definite lesion, with a spreading apart and splitting of the elastic lamellae (fig. 1 *B*). Fourteen rabbits were killed at the age of 1 month,

6 of them from mothers which had received viosterol and 8 from the 2 control mothers. Three of the former and 4 of the latter, or one half of the 14, presented lesions. At 8 months of age, practically all the animals in this stock had aortic changes. In general the older the animal the more marked were the changes, although deposits of calcium were observed in the young from both the viosterol treated and control mothers as early as at 3 months of age. The incidence of the lesions at various ages is graphically summarized in figure 3.

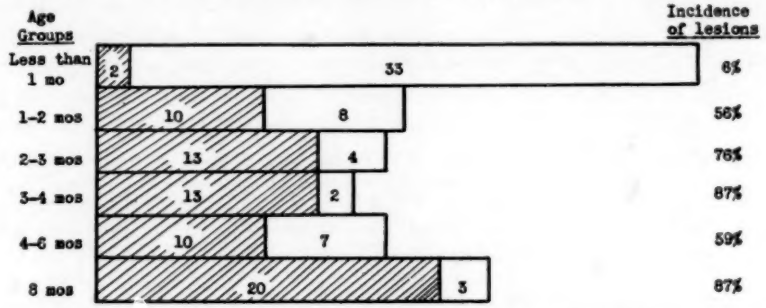

Fig. 3.—Incidence of aortic medial degeneration among young rabbits, classified according to age. In this and figure 4, the shaded areas represent the number of rabbits presenting lesions; the unshaded, the number with normal aortas.

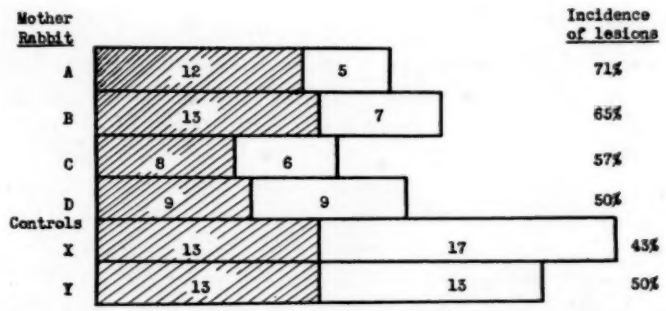

Fig. 4.—Incidence of aortic medial degeneration among young rabbits, classified according to the mother. Four mothers (*A, B, C* and *D*) received viosterol prior to the first breeding.

The incidence of the lesions in the young from the several mothers is summarized in figure 4. It ranges from 71 per cent in the young from mother A to 43 per cent in those from 1 of the control mothers. Reference to the table shows that all but 2 of the young from mother A were killed at ages of 1 month or more, which probably accounts for the high incidence in this family. The variations between the other 5 families are probably without significance. The total incidence in the entire group of 125 rabbits is 54 per cent.

At the end of the experiment, the mothers were killed and autopsies performed. The only significant lesions were in the aortas. Those from the 2 rabbits (A and B) which received 30,000 rat units of viosterol presented the greatest degree of change with numerous partly calcified small plaques in the arch. The lesions in rabbit C, which received 200,000 rat units, were somewhat less marked. No changes were demonstrable, even microscopically, in the aorta from rabbit D (200,000 units). The aortas of the 2 control mothers presented moderate changes, comparable to those in rabbit C. Thus no correlation is possible between the dose of viosterol, the amount of sclerosis in the mother's aorta and that in the aorta of the young. Although it is somewhat difficult to calculate in terms of rat units the doses of viosterol used by Junck [1] and by Schmidtmann (quoted by Junck), they seem to fall within the same range or somewhat under that which was used in this investigation.[4]

Junck gave a "total of 0.5 mg. of irradiated ergosterol during eight days, as standardized vigantol," probably a total dose of about 10,000 rat units. He gave also to another rabbit "2 drops of standardized vigantol."

The present attempt, then, to reproduce the transmission of sclerosis due to viosterol from mother to young must be deemed unsuccessful. All the lesions in the aforementioned animals are to be considered as spontaneous.

COMMENT

In the fifty-three years which have elapsed since Israel's [5] original description of medial degeneration and calcification in the rabbit's aorta, under the name of chronic endaortitis deformans, similar changes have been reported following a great variety of experimental procedures, including the use of bacterial, chemical and physical stimuli. Judging from the descriptions and illustrations, many of the lesions were entirely comparable to those now recognized to occur spontaneously in the rabbit's aorta. There was a wide quantitative variation in this recognition, however, ranging from none to 45.5 per cent of the rabbits. The literature has been largely summarized by Newburgh and Clarkson,[6] Dominguez,[7] Nuzum and his co-workers [8] and Benson and his co-workers.[9] The majority of observers saw spontaneous medial

4. Schmidtmann, as quoted by Junck,[1] gave "4 mg. of irradiated ergosterol daily for twelve days in the form of 10 drops of 1 per cent vigantol." According to the Winthrop Chemical Co., 1 per cent vigantol in 1929 was probably about 1,500 D (180,000 rat units per cubic centimeter).

5. Israel, O.: Virchows Arch. f. path. Anat. **86**:299, 1881.

6. Newburgh, L. H., and Clarkson, S.: Arch. Int. Med. **31**:653, 1923.

7. Dominguez, R.: Arch. Path. **5**:577, 1928.

8. Nuzum, F. R.; Elliot, A. H.; Evans, R. D., and Priest, B. V.: Arch. Path. **10**:697, 1930.

9. Benson, R. L.; Smith, K. G., and Semenov, H.: Arch. Path. **12**:924, 1931.

changes in less than 10 per cent of normal control rabbits. Dominguez found the mean for more than 3,500 rabbits examined during the preceding twenty years in this country and in Europe to be less than 6 per cent.

The earliest report of the recognition of aortic medial degeneration in normal rabbits appears to be that of Fischer,[10] in 1905, who established that lesions of the media of the aorta similar to those resulting from epinephrine may occur spontaneously. However, Kaiserling,[11] in 1907, in the course of a cautious evaluation of the then current enthusiasm for sclerosis from epinephrine stated that several years previously he had seen a calcified aorta in a stock rabbit. In 1907 Miles [12] drew down on herself a fire of comment by reporting from Colorado the occurrence of medial aortic lesions in 17 of 49 normal rabbits, that is, in 34.8 per cent. Miller,[13] in a letter in the same year, objected to Miles' conclusions, emphasized the failure of previous workers, including Jores,[14] to observe spontaneous lesions and expressed his belief that Miles' animals were unusual and probably associated with an abnormal environment. In the summarizing table published by Dominguez, Miles' figure of 34.8 per cent was conspicuous as the highest, with the next 15.4 per cent that from unpublished data by Goldblatt. Levin and Larkin,[15] 1910, were quoted as observing that 12.9 per cent of normal rabbits have spontaneous aortic lesions. But a perusal of the latter's paper reveals that of 240 rabbits "used for laboratory purposes mainly for obtaining blood serum, and with no toxic substances of any kind introduced," 31, or 13 per cent, had gross aortic lesions and were not examined further. The aortic arch of the remaining 209 animals was examined microscopically. Seventy-eight of the 209 rabbits, or 32.5 per cent of the original 240, presented microscopic lesions, making a total incidence of 45.5 per cent. Inasmuch as the exact handling of these animals was not specified, one cannot be certain that all of these lesions were spontaneous, but the significant point remains that many of them were noted only after microscopic study. Microscopic studies of the aorta in the several other normal series in the literature appear to have been limited to details of lesions noted grossly.

Of the 68 involved aortas in the series of 125 rabbits of this study, 10 had a normal gross appearance, the medial lesions being noted only in microscopic sections. In 7 other aortas the gross conditions were recorded as questionable and a definite diagnosis was possible only after microscopic examination.

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10. Fischer, B.: Verhandl. d. XXII Kong. f. inn. Med. **20**:235, 1905.
11. Kaiserling, C.: Berl. klin. Wchnschr **44**:29, 1907.
12. Miles, A. B.: J. A. M. A. **49**:1173, 1907.
13. Miller, J. L.: J. A. M. A. **49**:1789, 1907.
14. Jores, L.: Beitr. z. path. Anat. u. z. allg. Path. **41**:167, 1907.
15. Levin, I., and Larkin, J: Proc. Soc. Exper. Biol. Med. **7**:109, 1910.

The explanation of the high incidence of medial degeneration in the aortas of this series of rabbits and of the great differences in incidence reported from various laboratories is not obvious. Faulty diet does not seem to be a factor, as the diet used in this experiment was considered excellent. General living conditions were also ideal, except for relative lack of exercise and of direct sunshine. There is no apparent seasonal factor. Infection in the ordinary sense, including coccidiosis, can be excluded. A difference in the rabbit strain seems probable in view of the wide geographic differences in incidence. The observation of Zeek [16] that inbreeding may produce a strain relatively free from spontaneous sclerosis is very illuminating. The lesion pathologically appears to be a toxic degeneration. If this is true, it furnishes another example of the relationship of heredity and susceptibility to disease.

SUMMARY

A study was made of the aortas of 125 apparently normal young rabbits aged from 1 day to 8 months and of known parentage. They represented the successive litters of 4 female rabbits given viosterol prior to the first breeding and of 2 untreated female rabbits. Spontaneous medial degeneration, or "arteriosclerosis," was observed in 68 of the 125 rabbits or in 54 per cent. No significant difference was demonstrable between the young from the treated and the untreated mothers. Lesions were found at ages as early as 10 days. The incidence increased with age to 87 per cent at 8 months. Microscopic examination was necessary for a positive diagnosis in 25 per cent of the lesions.

16. Zeek, P.: Arch. Path. **16**:302, 1933.

POSSIBLE FUNCTIONAL SIGNIFICANCE OF THE LONGITUDINAL MUSCLE IN THE ADRENAL VEINS IN MAN

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The peculiar structure and arrangement of the longitudinal muscle bundles in the veins within the adrenal medulla in man must indicate a function of considerable importance. The observers who have studied these structures histologically have proposed theories which do not seem adequate. Since the ordinary laboratory animals show only slight development of these muscle bundles, the experimental attack on the problem of their function is impossible.

Recently Bauer, Dale, Poulsson and Richards [1] studied with great care and ingeniousness the control of the circulation through the liver in the dog and clearly demonstrated a mechanism determining the rapid outflow of blood which depends on the peculiar structure of the longitudinal muscle bundles in the hepatic veins of the dog. Since there is considerable similarity between these muscles in the hepatic veins of the dog and those in the adrenal veins in man, it seems altogether possible that the precise experimental results obtained by Bauer and his co-workers in explaining the functional significance of the hepatic veins in the dog can be applied to the consideration of the function of the adrenal veins in man. Without the possibility of direct experimental attack, the formation of a hypothesis depending on an analogy in structure seems justifiable.

In many adrenals removed from the human adult the following features may be recognized in sections prepared in a routine way from fixed tissues. The veins within the medulla in cross-section show substantial bundles of muscles running longitudinally along the length of the vein (fig. 1 *A*). These bundles occur at irregular points, and between the muscle bundles the wall of the vein is thin and lacking in muscle. The longitudinal bundles protrude markedly into the lumen and nearly touch the endothelium on the opposite side of the lumen. No circular muscle fibers are presented. Passing into these muscular veins are smaller blood vessels with very thin walls or merely with endothelium (fig. 1 *B*). As these vessels enter a larger vein, they pass

From the Department of Pathology, School of Medicine, University of Pennsylvania.

1. Bauer, W.; Dale, H. H.; Poulsson, L. T., and Richards, D. W.: J. Physiol. **74**:343, 1932.

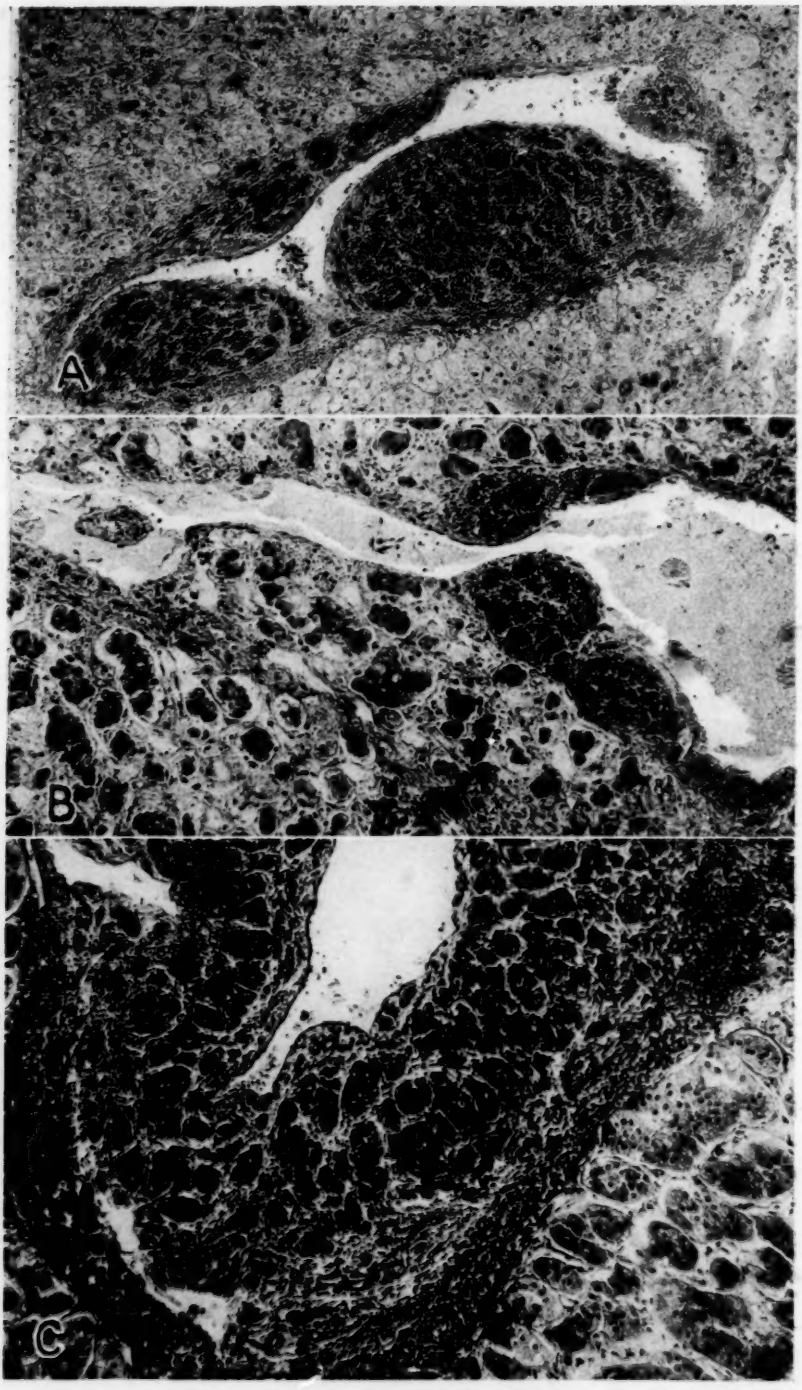

Fig. 1.—*A* shows longitudinal muscle seen in cross-section protruding into the lumen of a vein in the adrenal gland of a human being; *B*, the entrance of tributaries into human adrenal vein of medium size guarded by muscle bundles, and *C*, the main adrenal vein, with substantial bundles of longitudinal muscle completely surrounding the lumen.

between muscle bundles, so that the orifices are actually encroached on by the muscles in the larger vessels, and the obvious dilatation of the small vessels proximal to their entrance into the larger vein gives clear evidence that the muscle is actually causing functional obstruction to the tributaries. As the main adrenal vein is approached, the muscle bundles increase in size and surround the lumen almost symmetrically (fig. 1 *C*). The larger hepatic veins of the dog (fig. 2 *A*) are similar in structure to the main adrenal vein. The peculiar asymmetrical arrangement of muscle in the smaller tributaries of the adrenal vein in man is a development far beyond anything seen in the hepatic veins of the dog. According to Popper,[2] however, if the dog's liver is fixed at a time when the muscle in the hepatic veins is contracted, projections into the lumen are seen, so that in cross-section the lumen appears star-shaped. This effect is shown in figure 2 *B*. In figure 2 *C* are seen longitudinal muscles extending the length of two hepatic veins which are tributaries to the vena cava of the dog.

Before considering the application of the results obtained by Bauer to the adrenal in man, a summary is presented of the previous suggestions as to the significance of the musculature of the adrenal veins. Ferguson[3] stated that "the peculiar longitudinal arrangement of the muscular tissue, the valve-like protuberances at the junctions of the venous vessels, the absence of circular muscle from the walls of the veins of all sizes, and the general appearance of these vessels which are so remarkably different from the veins of most other organs, become, to say the least, extremely significant of a close structural relation, physiologically speaking, to the presence of an astringent secretion in the outflowing blood current." He offered, however, no explanation of how these mechanical features are effective.

Poll[4] expressed the belief that the musculature is a means of securing a smooth and equal outflow of blood. Peindarie[5] maintained that the muscles of the medium-sized branches act as valves obstructing the flow from the smaller to the larger veins and that the powerful musculature expresses the contents of the larger veins into the general circulation. Maresch,[6] from serial sections and wax reconstructions, concluded that when the longitudinal muscles contract the thickening and shortening of the fibers result in narrowing or complete closure of the lumen. At the points at which the smaller tributaries pierce the muscle bundles in reaching the lumen of the larger vessel, contrac-

2. Popper, H.: Klin. Wchnschr. **10**:2129, 1931.
3. Ferguson, J. S.: Am. J. Anat. **5**:63, 1906.
4. Poll, H.: Berl. klin. Wchnschr. **46**:1973, 1909.
5. Peindarie, J.: Compt. rend. Soc. de biol. **83**:958, 1920.
6. Maresch, R.: Wien. klin. Wchnschr. **34**:44, 1921.

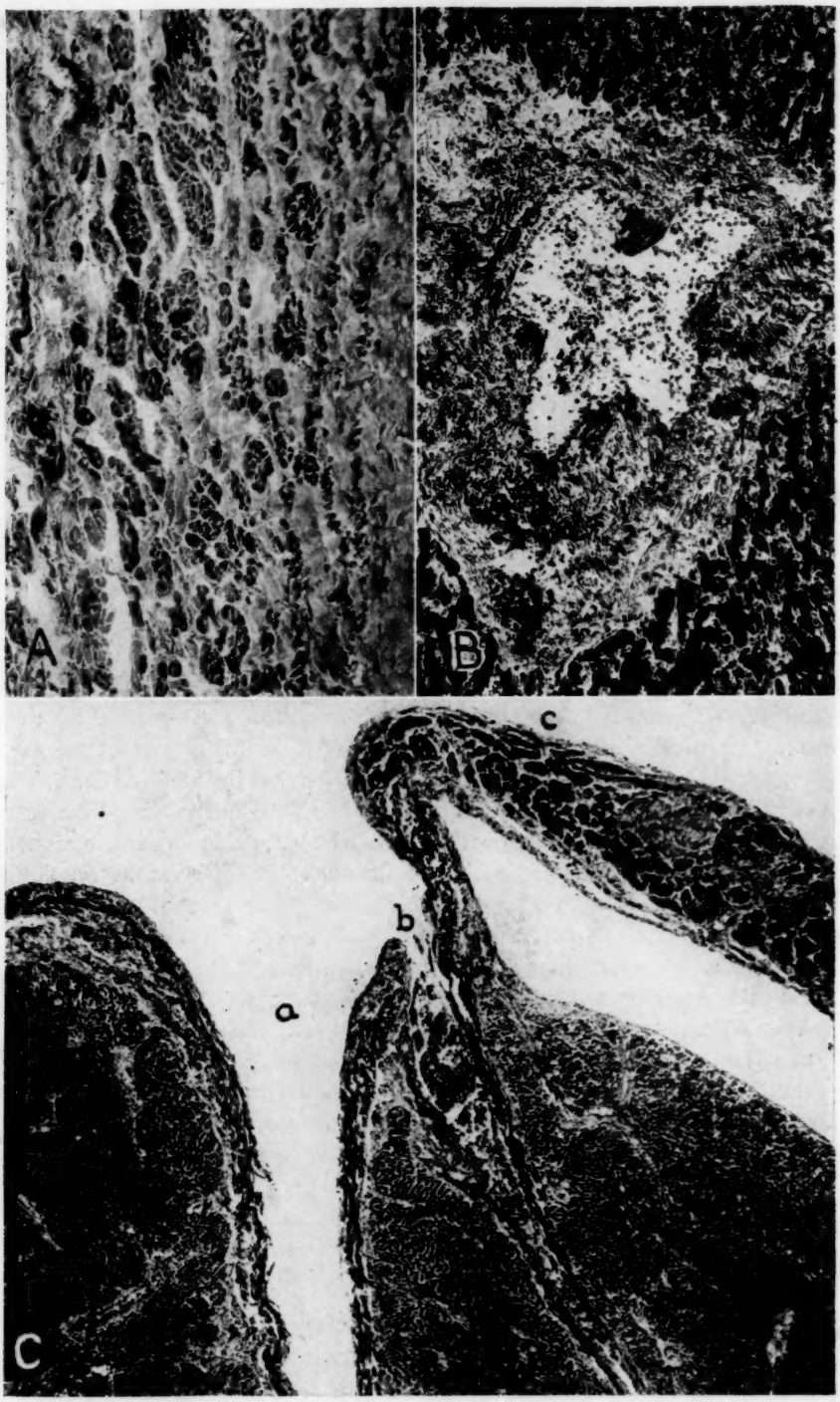

Fig. 2.—*A* shows bundles of longitudinal muscle seen in cross-section in a large hepatic vein of a dog; *B*, a cross-section of a small hepatic venous tributary in a dog with a stellate lumen and bundles of longitudinal muscle in the four pillars which project into the lumen, and *C*, longitudinal muscles seen in longitudinal section in two hepatic veins (*a*) and (*b*). The muscles are continuous with the longitudinal muscle seen in cross-section in the vena cava (*c*). Mallory's differential connective tissue stain.

tion of the muscle holds back the contents of the smaller vessel, which is seen to undergo sinuous widening. Under stimulation of the sympathetic nerve supply, contraction of the muscles hinders outflow of the blood and thereby enriches its epinephrine content which is available for subsequent discharge. Maresch, however, suggested another explanation, namely, that by contraction of the musculature the organ is squeezed as a sponge in the direction of the central vein. Against this theory is the fact that the muscle bundles become larger as the main vein is approached, so that shortening and thickening of the muscles during contraction should cause progressively more complete occlusion of the lumen as the hilus is approached.

Kutschera-Aichbergen [7] accepted Maresch's view that the muscles in the central vein block the tributaries and cause damming of the blood in the medulla. Maresch had suggested that the blood held back by the contracted veins might find its way into anastomoses outside the adrenal gland. Kutschera-Aichbergen enlarged on this idea, and, by injection of a dye into the left adrenal vein, he observed that the dye passed through anastomoses of the capsular vessels into the pancreas and liver. He therefore expressed the belief that with contraction of the muscle in the adrenal veins the current of blood is reversed and epinephrine is forced through the anastomoses into the portal circulation. He stated that epinephrine injected into the portal circulation is more effective in producing hyperglycemia than when introduced into other veins, and, moreover, that epinephrine introduced into the portal vein does not raise the blood pressure as it does when introduced into other veins. He assumed that the muscles in the adrenal veins contract under stimulation of the sympathetic nerves.

Henderson,[8] from the study of wax reconstructions, stated that when two veins approach the point of anastomosis the longitudinal fibers in each vessel "fuse in the angle of anastomosis." He expressed the belief that the "significance of this peculiar muscular arrangement in the wall of the central veins of the suprarenal gland probably consists in the fact that contraction of the muscles results in active dilatation of these veins." His conception is best understood by consulting his diagram. He suggested that "this mechanism regulates the outflow of blood" under sympathetic nervous stimulation. This explanation can apply only to cases in which each vessel, before joining the other, contains muscle; it does not explain what happens as the result of frequent entrance of vessels containing no muscle into larger veins with muscle surrounding the entrance of the smaller vessel.

7. Kutschera-Aichbergen, H.: Frankfurt. Ztschr. f. Path. **28**:262, 1922.

8. Henderson, E. F.: Anat. Rec. **36**:69, 1927.

Goldzieher and Sherman [9] measured the thickness of muscles in material obtained in fifty-one autopsies selected at random but limited to those performed on adults, and he calculated the ratio of the thickness of the muscle to the outer diameter of the vessel. The ratios were greater in cases of hypertension and in those of "renovascular alterations without manifestations of hypertension." They stated: "It seems reasonable to assume that such hypertrophy is the result of constant overexertion resulting from the attempt to regulate and hold back the excessive liberation of the pressor glandular discharge." The investigators claimed that their results added "new and weighty evidence to the theory which links hypertension and allied diseases with functional disturbances of the suprarenal glands." In their measurements of muscle there seems to be an error in the unit used for the statement of absolute values. The values of from 3.1 to 6.1 microns for the thickness of hypertrophied muscle are obviously too low. However, their relative grouping of musculature as normal and hypertrophied holds in spite of this.

Allen [10] measured by means of a planimeter the muscles and lumens of the veins in eighteen patients with hypertension (average age, 54) and in twenty-five persons in whom the blood pressure was normal (average age, 48). He did not state the absolute measurements, but the ratio of muscle area to the area of the lumen was twice as great in the cases of hypertension. He expressed the belief that the muscular hypertrophy "in all probability indicates increased functional activity, which occurs as a result of sympathetic overactivity."

Allen and Page,[12] assuming that the inferior vena cava has the same intravenous pressure as that of the adrenal veins, measured the thickness of the muscle in the inferior vena cava and observed that it was not increased in patients dying with increased venous pressure. They concluded that the muscular hypertrophy of the adrenal veins in patients with hypertension was not caused by increased intravenous pressure.

Kashiwagi [13] observed that the muscles of the adrenal veins were hypertrophied in diseases in which he stated that there is an increased epinephrine content of the blood, such as contracted kidney, and that they were less hypertrophied in conditions in which he claimed that there is a decrease in the epinephrine content; but proof is lacking that there are such changes in the epinephrine content of the blood in the conditions he mentioned. Kutschera-Aichbergen [7] suggested that blocking of the outflow of blood by the musculature of the veins with

9. Goldzieher, M. A., and Sherman, I.: Arch. Path. **5**:1, 1928.
10. Allen, E. V.: Ann. Int. Med. **3**:153, 1929.
11. Deleted.
12. Allen, E. V., and Page, I. H.: Deutsches Arch. f. klin. Med. **168**:193, 1930.
13. Kashiwagi, S.: Tr. Jap. Path. Soc. **12**:154, 1922.

subsequent engorgement of the capillaries may be responsible for certain cases of adrenal hemorrhage. Kraus,[14] in discussing adrenal hemorrhage in adults, stated that certain cases may be due to trauma inducing sudden spastic contraction of the muscles of the veins, with rupture of the vessels that have been overdistended by this blockage.

Many of these theories of the function of the venous musculature assume that the muscles of the veins contract under sympathetic stimulation and that contraction aids the outflow of epinephrine. There are a variety of proofs of the outpouring of epinephrine, which is known to occur rapidly under sympathetic stimulation in various emergencies. But it is difficult to visualize the mechanism by which contraction of bulky muscles can increase venous outflow. In fact, there is every indication from microscopic sections that contraction by thickening the muscles occludes the lumen and hinders outflow. From sections it is clear that at the point at which a tributary enters a larger vein between muscle bundles it is obstructed and dilates proximally and that this thin-walled dilatation is structurally suited to act as a reservoir (fig. 1 *B*). Allen [10] observed that the total area of the lumens of all veins increased in the adrenals in which the muscle of certain veins showed hypertrophy. From microscopic sections it can also be seen that the longitudinal muscle bundles appear to occlude the lumen more completely than would be possible if there were merely a circular arrangement of muscle fibers. Even with the maximum constriction of a structure controlled by circular fibers, there still remains a lumen, but when longitudinal muscle bundles placed only at certain points of the wall of the vein thicken by contraction the pliability of the delicate wall at all other points makes it possible for the endothelium on the opposite side of the lumen to fit closely over the protruding muscle. The surrounding tissue apparently offers more resistance than the blood in the lumen, as in all the sections the muscle bulges into the lumen rather than indents the surrounding tissue. The theory suggested in this investigation for the functional significance of the muscles in the adrenal veins seems to be implied in the work of Popper,[2] who described what he called the throttle mechanism of the hepatic veins in the dog. He was led to this study by the recognition of the similarity in structure to that of the adrenal veins.

When muscle hypertrophies one has a right to believe that it does so in response to the demand for increased functional activity. But in what way is contraction of hypertrophied muscle effective in this case? Is it possible that these muscles are in a state of more or less

14. Kraus, A.: Frankfurt. Ztschr. f. Path. **43**:372, 1932.

constant partial contraction or tonus under normal conditions, which hinders the outflow of blood and keeps the epinephrine content of the blood high within the adrenal as a reservoir, and that sympathetic stimulation under conditions of emergency relaxes the contracted muscles and permits the rapid passive release of blood containing a high concentration of epinephrine from the adrenal gland? Although such an idea seems radical at first glance and diametrically opposed to most of the aforementioned theories, this is exactly what Bauer and his co-workers [1] demonstrated experimentally in the outflow of blood from the hepatic veins in the dog. This phenomenon depends on a musculature in the vein similar to that of the adrenal vein in man. They showed in the isolated perfused liver, in which records were made of the pressure of the blood in the hepatic artery and portal vein and of the volume of the liver, that either small doses of epinephrine comparable to those liberated physiologically or stimulation of the splanchnic nerve greatly increased the outflow of blood from the hepatic veins through the vena cava, in spite of lessened inflow. This action is determined by a mechanism like a sluice located near the caval orifice of the main hepatic veins, which consists of longitudinal muscle bundles in the walls of the veins. Normally the tonus hinders the outflow of blood from the liver. With histamine the muscles contract; with epinephrine or with sympathetic stimulation, they relax, and therefore the outflow is increased. The mechanism permits the accumulation of a reserve in the liver and a release into the general circulation under special conditions. Bauer's paper should be consulted for details of the mechanism and for a review of the previous work bearing on this subject.

Grab, Janssen and Rein,[15] at about the same time, arrived independently at conclusions nearly similar to those of Bauer and his co-workers, and their experiments have the advantage of having been done under more natural conditions. Furthermore, they showed that atropine increased the venous outflow. It seems probable that the effect of atropine indicates that the muscle is normally in a state of partial contraction under parasympathetic stimulation and that relaxation is produced by such a parasympathetic depressant as atropine.

It seems justifiable to attempt an application of these experimental observations on the circulation in the liver of the dog to that in the adrenal in man, since a direct attack on the problem is impossible in the case of the adrenal veins. In many cases in which sympathetic stimuli are known to be effective, they may be opposed by parasympathetic stimuli.

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15. Grab. W.; Janssen, S., and Rein, H.: Ztschr. f. Biol. **89**:324, 1929; Klin. Wchnschr. **8**:1539, 1925.

One may therefore theorize about the possible effect of parasympathetic innervation of the adrenal, in which the powerful effects of sympathetic stimulation are known. Parasympathetic fibers are known to innervate the adrenals, but their action is not understood. It is conceivable that parasympathetic impulses are responsible for keeping the muscles of the adrenal veins, when these are well developed, under a certain amount of contraction under ordinary circumstances. In consequence, the secreted epinephrine accumulates in the smaller veins and capillaries of the medulla in high concentration. When a sudden emergency arises, sympathetic stimuli relax the muscles of the veins (just as bronchial muscles relax under sympathetic stimulation), and there is a sudden release of blood of high epinephrine content. The suddenness of the release of large quantities of epinephrine which is known to occur under sympathetic stimulation is, in all likelihood, a vasomotor release of secretion held in reserve rather than entirely the effect of the nervous stimulation of cells to hypersecretion, as is often considered the case.

As long ago as 1897, Biedl [16] maintained that the increased outflow of blood from the adrenal on sympathetic stimulation was due to active dilatation of the "adrenal vessels" by fibers in the splanchnic nerve producing dilatation. Burton-Opitz and Edwards [17] confirmed Biedl's statement and remarked: "It also seems singular that the excitation of the splanchnic nerve . . . should dilate the blood vessels of the suprarenal gland and constrict those of the other organs innervated by this nerve." Rigler and Rothberger [18] stated that the blood vessels of the adrenal do not contract as a result of the stimuli that ordinarily contract vessels, such as splanchnic stimuli, while substances which ordinarily produce dilatation of blood vessels also dilate the adrenal vessels. In all these pharmacologic experiments the authors do not specify the type of "blood vessels." Yet is not absence of constriction in the veins necessary in order that arterial dilatation may effect a greater outflow?

Feldberg, Minz, and Tsudzimura [19] recently described liberation of acetylcholine from the adrenals of the cat during stimulation of the splanchnic nerve. In this investigation they were not concerned with a vascular mechanism within the adrenals controlling the discharge of the cellular secretion. But from the point of view of the present study,

16. Biedl, A.: Arch. f. d. ges. Physiol. **67**:443, 1897.

17. Burton-Opitz, R., and Edwards, D. J.: Am. J. Physiol. **43**:408, 1917.

18. Rigler, R., and Rothberger, C. J., in Bethe, A., et al.: Handbuch der normalen und pathologischen Physiologie, Berlin, Julius Springer, 1927, vol. 7, pt. 2, p. 1028.

19. Feldberg, W.: Minz, B., and Tsudzimura, H.: J. Physiol. **81**:286, 1934.

their data on the liberation by the adrenal of acetylcholine is of considerable interest in view of the known relation of acetylcholine to parasympathetic activity.

If the studies on the adrenals of animals apply to those of man, the manner in which epinephrine is discharged under sympathetic stimulation becomes clear, but the added factor of venous obstruction in man must be considered. In man, with bulky muscles in the adrenal veins, a check on the rapid discharge of epinephrine occurs which does not exist in animals.

There remains to be explained the significance of the hypertrophy of the muscles in the adrenal veins which has consistently been noted in patients with hypertension. There is no clinical or experimental evidence that patients with hypertension have hyperepinephrinemia. To consider that hypertrophy of the adrenal musculature indicates increased discharge of the secretion of epinephrine in patients with hypertension is therefore illogical. Furthermore, on this supposition, if hypertensive patients did have hyperepinephrinemia they would have changes in carbohydrate metabolism, which is not true. May not the hypertrophied muscle of the adrenal veins in a patient with hypertension be a protective phenomenon inhibiting the outflow of epinephrine during episodes of sympathetic stimulation in a person whose blood pressure is already dangerously high? Such a delicate balance between opposing sympathetic and parasympathetic stimuli is known to be effective in averting crises in other organs, graphic illustrations of which have been given by Cannon.[20] He stated that "when a factor is known which can shift a homeostatic state in one direction it is reasonable to look for automatic control of that factor or for a factor or factors having an opposite effect." In other words, it is now suggested that the hypertrophy of the musculature in the adrenal is a protective phenomenon, occurring as a result of hypertension rather than being causative, a compensating contraction opposing sympathetic activity. In this connection, it was interesting to find a case of extensive development of the muscle of the adrenal veins in a young adult dying of exophthalmic goiter, in which it is known that there is intense activity of the sympathetic nervous system.

Animals are usually free from hypertension and also show an almost negligible development of muscle bundles in the adrenal veins. May it not be possible that in the absence of hypertension there is no compensatory hypertrophy of muscle in the medium-sized veins limiting the outflow of excessive secretion of epinephrine in animals? It is known that in the adrenal gland of the infant the main adrenal vein contains muscle but that the medium-sized veins within the medulla

20. Cannon, W. B.: Physiol. Rev. **9**:399, 1929.

are almost lacking in muscle. This again fits in with the theory that the development of conspicuous muscle bundles is a gradual compensating hypertrophy.

Apparently no quantitative studies have been made of the age at which this hypertrophy takes place.

PRESENT INVESTIGATION

An attempt has been made to study the development of these muscles with age. Adrenal glands obtained in autposies performed as a routine in the department of pathology were studied at random, with the exclusion of those in which the medulla showed postmortem softening and lesions such as tuberculosis or tumors. One adrenal was studied in each case. In most cases the tissue fixed in Zenker's solution was stained with Mallory's connective tissue stain in order to differentiate the muscle from the connective tissue with certainty. Approximate measurements were made of the area of the muscle in cross-sections of veins. As most of the bundles were in the form of ellipses, occasionally of circles, the long (a) and short (b) diameters were measured by means of an ocular micrometer and the area calculated as $\frac{a}{2} \times \frac{b}{2} \times \pi$. The values for all the areas of muscle in a single vein were added. Selection was made of the largest venous branch lying within the medulla which showed asymmetrical arrangement of muscle (in order to exclude the main vein, which is symmetrically surrounded by muscle), and vessels with the area of the lumen larger than 0.35 sq. mm. were excluded. The veins which happened to be seen in longitudinal section were also excluded. The measurements were, of course, merely approximations, but at least they gave a more objective means of comparison than does a classification on the basis of 2 plus, 3 plus, etc. Without serial sections, the largest vein probably was often missed. Consequently, the recording of the absence of musculature or of muscles of small area is of no significance. In cases in which the muscles were well developed, the bundles were obvious in any section. Attention was concentrated on determining at what age muscular development could be noted. Therefore, the observation of well developed muscle at 20 years of age was of more significance than that of little muscle at 50. A chart was constructed (fig. 3) which in no sense is intended as an exact quantitative record. It shows, however, that, within the limitations of accuracy mentioned, it is only in the later age periods that well developed muscles can frequently be found. Certain low values observed in those periods may merely indicate that in the single section studied little muscle was noted rather than that the adrenal veins of that person contained no muscle. The range in, not the absolute, values at the differ-

ent age periods is of concern. In the material obtained in one hundred and seven autopsies selected at random, it was observed that there was a preponderance of persons over 35. Thereupon, supplementary material was selected from the adrenals of persons under 35 and included in the general series. Sections from these tissues

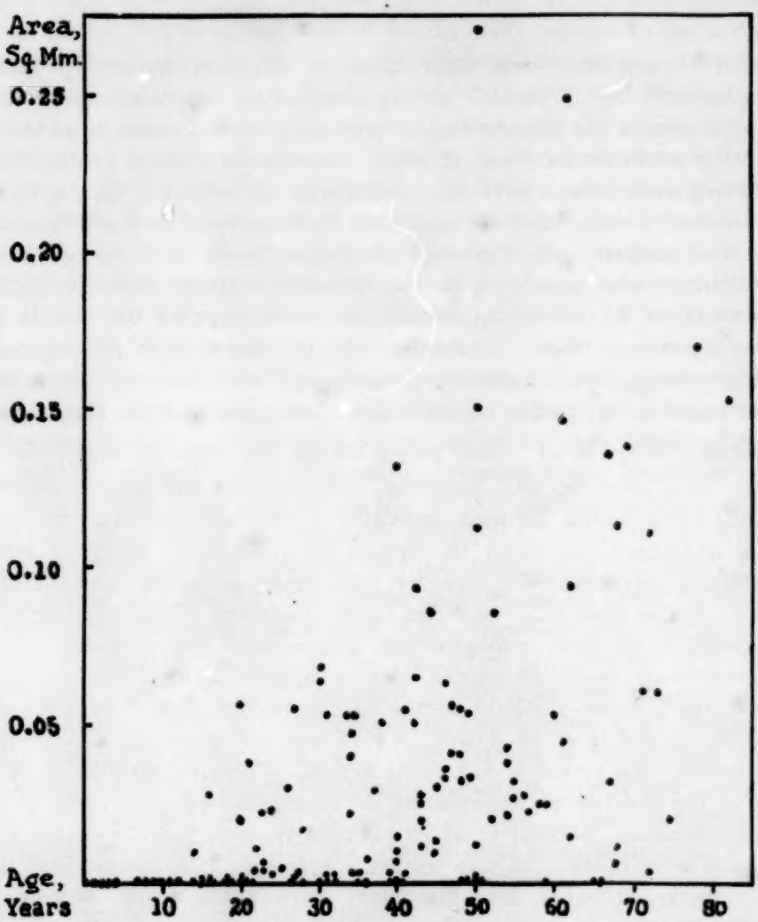

Fig. 3.—Areas of cross-sections of muscle in veins of medium size (excluding the veins with the area of the lumen exceeding 0.35 sq. mm.). The single dot in the space representing the 1 year period indicates the average value for the areas obtained in twenty-six children, ranging from new-born infants to those of 11 months. Other single dots in the early age periods usually represent the values obtained for more than one child. After the 12 year period each dot represents the value determined for a single person.

were stained with hematoxylin and eosin instead of with Mallory's connective tissue stain. The total number of cases studied was 172.

SUMMARY

A theory is proposed to explain the rôle which the longitudinal muscles in the adrenal vein in man play in controlling the discharge of epinephrine. It is suggested that under sympathetic stimulation these muscles relax and widen the lumen and that under parasympathetic stimulation they contract and, by their consequent thickening, occlude the lumen.

In the material obtained in a series of autopsies, it was observed that these muscles developed with age.

It is suggested that the hypertrophy of these muscles may be a protective phenomenon, checking the discharge of epinephrine in hypertensive patients.

HORMONAL ORIGIN OF ENDOMETRIOMA

A HYPOTHESIS

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Endometriosis is a term which was introduced by Sampson to include a variety of adenomatous lesions of the female pelvis the histologic and functional characteristics of which are identical with those of the endometrium. Until 1896 endometriosis was considered to have its origin in the müllerian duct, but in that year, owing to the influence of von Recklinghausen, the wolffian system was considered to be the original source. Then came the intervention of Cullen, who firmly established the theory of the origin of adenomyoma from mature endometrium. To Russell belongs the credit of being the first to report the presence of aberrant endometrium in the ovary, a condition originating, in his opinion, from inclusion of the müllerian duct. Since the appearance of Sampson's studies in 1921 this subject has been favored with renewed interest, and at present no gynecological study receives more lively discussion than that of endometriosis.

In Sampson's opinion endometrioma is second only to uterine myoma in frequency. In 119 cases he found the sites of the endometriomas to be as follows: ovaries, 110; uterus, 64; rectovaginal septum, 6; peritoneum, 2, and the abdominal wall in a laparotomy scar, 1. In addition to these locations endometriomas have been found in or on the majority of the pelvic organs.

The etiology of endometrioma is still a matter of debate. Up to the present credit must be given (1) to Cullen [1] for his theory of the mucosal or diverticular origin of diffuse uterine growths; (2) to Ivanoff [2] for his serosal theory, which draws attention to the proliferative potentialities of the peritoneal mesothelium; (3) to Sampson [3] for the hypothesis of tubal regurgitation, or the theory of retrograde menstruation with the direct implantation of living endometrial grafts, and (4) to Robert Meyer,[4] championed in this country by Novak, for his invaluable demonstration of epithelial heterotopy and metaplasia, which helps to render more intelligible the occurrence of many of the extrapelvic growths of like character. Suffice it to say that at present two views are generally

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1. Cullen, T. S.: Adenomyomata of the Uterus, Philadelphia, W. B. Saunders Company, 1908.
2. Ivanoff, N. S.: Monatschr. f. Geburtsh. u. Gynäk. **7**:295, 1898.
3. Sampson, J. A.: Arch. Surg. **3**:245, 1921.
4. Meyer, R., and Kitai, I.: Zentralbl. f. Gynäk. **48**:2449, 1924.

held as to the cause of these tumors: either Sampson's hypothesis of cellular spill or the theory of heteroplasia of a serosal cell, advocated as early as 1898 by Ivanoff. However, that neither of these theories can explain the occurrence of endometrioma at all the known sites is generally accepted. The theory of heteroplasia of the serosal cell cannot explain the perineal endometrioma, while endometriosis of the inguinal canal is difficult to ascribe to the theory of the implantation of fragments of the uterine lining.

It is not the intention in this paper to enter this field of discussion, and the following remarks and observations will hold good irrespective of the primary source of the endometrial elements. If the endometrioma is explained by the theory of implantation, there must be some stimulant or environment which determines whether the endometrioma will grow or perish. Sampson [5] took cognizance of this fact and stated that "the escaping cells need suitable soil." If, on the other hand, the origin of the endometrioma is from heteroplasia of serosal or germinal cells, some factor must be present which first initiates the cellular change and subsequently stimulates the endometrial growth. The actual origin of the endometrioma is unimportant, whether from implantation of a living graft or from cellular metaplasia; the all-important question is the determination of the cause of the igniting factor which controls the cellular change or which stimulates the endometrial implant to proliferation.

The hypothesis on which this paper is based is that the fundamental igniting cause has its origin in the excessive stimulation of the aberrant tissue by the ovarian follicular hormone. That the action of this hormone is not confined to the uterine endometrium alone, as demonstrated by the endometrial changes during the normal menstrual cycle, but influences the genital tract as a whole is easily proved.[6] When this hormonal action on the endometrium is abnormal, however, causing endometrial hyperplasia, it is equally abnormal in its action on ectopic endometrial tissue and causes, by means of cellular metaplasia of the potential serosal cells or by tumor proliferation of an aberrant endometrial implant, the formation of an endometrioma.

Through the work of Schroeder [7] and Meyer [8] in Germany, Shaw [9] in England and Graves,[10] Fluhman,[11] Novak,[12] Martzloff [13] and

5. Sampson, J. A.: Surg., Gynec. & Obst. **38**:287, 1924.
6. Estrogenic Substances: Theelin, report of the Council on Pharmacy and Chemistry, J. A. M. A. **100**:1331, 1933.
7. Schroeder, R.: Arch. f. Gynäk. **27**:102, 1915.
8. Meyer, R.: Arch. f. Gynäk. **93**:259, 1920.
9. Shaw, W.: J. Obst. & Gynec. Brit. Emp. **36**:1, 1929.
10. Graves, W. P.: Am. J. Obst. & Gynec. **20**:500, 1930.
11. Fluhman, C. F.: Surg., Gynec. & Obst. **52**:1051, 1931.
12. Novak, E., and Martzloff, K. H.: Am. J. Obst. & Gynec. **8**:385, 1924.
13. Martzloff, K. H.: Northwest Med. **33**:263, 1934.

Burch [14] in this country, the cause of endometrial hyperplasia has been thoroughly investigated. That the persistent and excessive stimulation of the ovarian follicular hormone, in the absence of any corpora lutea and with a possible anterior hypophyseal action in the background, is the cause of endometrial hyperplasia has been established by these observers. Burch [14a] and his co-workers produced endometrial hyperplasia in spayed mice by injection of the estrogenic principle, while Hofbauer,[15] by implantation of the substances of the anterior lobe or by injection of anterior hypophyseal extracts, demonstrated endometrial changes similar to those of endometrial hyperplasia. The general acceptance is at present that endometrial hyperplasia is the result of the unopposed and continued action of an excessive amount of the estrogenic principle derived from the multiple follicular cysts of the ovary.

The morphologic and functional characteristics of an endometrioma and of the uterine endometrium are similar; the integrity and function of the endometrioma are dependent on the presence of active ovarian tissue, since castration causes regression of the tumor; the endometrioma presents decidual reaction during pregnancy; it undergoes the phases of the menstrual cycle, changes dependent on the ovarian hormones, and, as proved by Gleave [16] in rabbits, the presence of the estrogenic principle is essential for the maintenance of the lesion.

Since the ovarian follicular hormone is the cause of endometrial hyperplasia and since the histologic structure of the endometrioma and that of the uterine endometrium are similar, it is logical to deduce that the igniting factor of endometrioma which brings about the cellular metaplasia or the proliferation of the endometrial implant is the estrogenic principle. That such is the case is all the more firmly established by the fact that in many instances the endometrioma presents hyperplasia which is typical histologically of endometrial hyperplasia and also by the high incidence of the association of endometrioma with uterine endometrial hyperplasia. The frequent finding of all the features of endometrial hyperplasia in an endometrioma, accompanied with similar changes in the uterine mucosa, indicates that the endometrioma can be caused only by the factor which determines the mucosal changes—the ovarian follicular hormone.

A distinction between endometrial hypertrophy or hyperplasia and the formation of a true tumor should be made. In hypertrophy or hyperplasia the etiologic factor is an abnormally high concentration of the ovarian follicular hormone in the circulation, acting apparently on

14. Burch, J. C., et al.: (*a*) Surg., Gynec. & Obst. **53**:338, 1931; (*b*) Arch. Path. **17**:799, 1934.

15. Hofbauer, J.: Surg., Gynec. & Obst. **52**:222, 1931.

16. Gleave, H. H.: J. Path. & Bact. **33**:675, 1930.

normally susceptible tissue. On the other hand, in the formation of a tumor, as in a case of endometrioma, the increased amount of the estrogenic principle in the blood acts on a hypersusceptible tissue which has the capacity to concentrate the hormone at the site of the tumor and to react by proliferation.

In a former contribution [17] on the relationship between endometrial hyperplasia and uterine fibroids, the high incidence of ovarian endometrial implants (30 per cent) was noted. At that time the suggestion was made that these three conditions might possibly have a common etiologic background. These figures have been rechecked and combined with recent observations, and it has been found that ovarian and uterine endometriomas were associated with endometrial hyperplasia and uterine fibroids in 64 per cent of the 44 cases studied, a figure far too high to indicate a mere coincidence.

Analysis of 44 Cases of Endometriosis

Type of Operation	No. of Cases
Hysterectomy	44
Bilateral salpingo-oophorectomy	18*
Removal of all remaining ovarian tissue	10*
Unilateral salpingo-oophorectomy	16
Condition of the Myometrium	
Fibromyomatous	44
Hyperplastic	24
Fibrotic	6
Adenomyomatous	8
Normal	15
Condition of the Endometrium	
Hyperplastic	40
Premenstrual	4
Condition of the Ovaries	
Multiple follicular cysts	44
Corpus luteum (mature)	4
Miscellaneous	
Salpingitis	18
Adhesions	20
Ovarian endometrial transplants	22†

* All ovarian tissue was removed in 28 cases (64 per cent).

† Fifty per cent of the total; in combination with the 8 adenomyomas, 64 per cent of the total.

Other authors likewise have called attention to the frequent association of endometrial hyperplasia, endometrioma and uterine fibroids. In

17. Witherspoon, J. T.: Surg., Gynec. & Obst. **56**:1026, 1933; Endocrinology **18**:703, 1933.

Jeffcoate's [18] series of 113 cases of endometriosis 79 women (71 per cent) presented endometrial hyperplasia, while 31 (28 per cent) presented uterine fibroids. Allen,[19] in his study of endometrioma, was impressed with the high incidence of the association of this condition with menstrual irregularities due to endometrial hyperplasia (70 per cent) and uterine fibroids (41 per cent) and the prevalence of relative sterility (60 per cent). Smith,[20] in 159 cases of endometrioma, noted associated endometrial hyperplasia in 42 per cent and uterine fibroids in 52 per cent of the patients.

Not only the uterine and the aberrant endometrium is stimulated to hyperplasia and tumor proliferation by the estrogenic principle, but the uterine musculature also is affected.[21] Because uterine fibroids are slow-growing tumors, the stimulation of the ovarian follicular hormone must be effective over a prolonged period in order that the formation of the fibroid can occur. Geschickter, Lewis and Hartman [22] have gone a step further in describing the action of the estrogenic principle. Their conclusion seems to indicate that gynecomastia in the male and virginal hypertrophy and the formation of fibro-adenoma in the female breast are dependent on the pathologic variations in the action of the ovarian follicular hormone on the epithelium of the mammary ducts; and that prolonged and interrupted stimulation by the estrogenic principle rather than brief, high concentration is necessary for the production of these abnormal conditions of the breast.

It therefore seems logical to deduce that the multiple follicular cysts of the ovaries, in the absence of corpora lutea, which cause, through the action of the estrogenic principle, endometrial, uterine and mammary hyperplasia or hypertrophy, likewise cause endometriomas, uterine fibroids and mammary fibro-adenomas.

There are two clinical features—functional uterine hemorrhage and sterility—that are associated with endometrioma, and their occurrence is explainable by the hypothesis just stated. Menstrual irregularities frequently accompany endometrioma, yet the source of the bleeding cannot, in many cases, be ascribed to the endometrial tumor. However, such hemorrhage can easily be explained by the associated endometrial hyperplasia of the uterine mucosa. Likewise the high frequency of sterility (60 per cent) [19] with endometrioma is due to the presence of the multiple follicular cysts of the ovaries in the absence of ovulation and of the formation of corpora lutea.

18. Jeffcoate, T. N. A.: J. Obst. & Gynec. Brit. Emp. **41**:684, 1934.
19. Allen, E.: Am. J. Obst. & Gynec. **26**:803, 1933.
20. Smith, G. V.: Am. J. Obst. & Gynec. **27**:806, 1929.
21. Witherspoon, J. T.: Surg., Gynec. & Obst. **57**:87, 1934; footnote 17.
22. Geschickter, C. F.; Lewis, D., and Hartman, C. G.: Am. J. Cancer **21**: 828, 1934.

CONCLUSION

The hypothesis that all forms of overgrowth (hyperplasia, hypertrophy or tumor proliferation) of the uterine endometrium and musculature, of the aberrant endometrioma and of the mammary glands are due to the same factor—the estrogenic principle—not only is supported by clinical and pathologic data but it explains satisfactorily the simultaneous development of these conditions and their associated clinical features—uterine hemorrhage and sterility.

TRANSPLANTATION OF SKIN AND CARTILAGE IN CHICKENS

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In a series of investigations we studied the transplantation of mammalian tissues; for this purpose, in the majority of cases, we used thyroid gland and cartilage with the adjoining tissues: parathyroid gland and fat, bone and muscle tissues. Our principal aim in these experiments was to analyze the relations which might exist between the organismal differentials of the host and transplant and the mode and intensity of the reactions of certain host cells against the transplant, as well as to ascertain the degree of toxicity of the body fluids of the host for the grafted tissues. Definite relationships were observed to obtain between these two sets of conditions: the character of the organismal differentials of the host and transplant and the effects of the host on the graft. We thought it of interest to extend these investigations to birds, in order to determine whether we should find here, also, similar connections between these two factors. Some experiments concerning transplantation of normal tissues in adult birds had already been made by several investigators (Loeb and Addison;[1] Schultz;[2] Danforth[3]), but these seemed as yet insufficient to allow us to arrive at definite conclusions so far as the problem stated here was concerned.

In regard to the technic to be applied in such investigations in birds and particularly in chickens, we suffered from a rather serious disadvantage as compared with one who undertakes similar experiments in mammals such as guinea-pigs, rats and mice. In the latter, one can readily use the aforementioned organs, which in their totality represent a sufficiently varied selection of tissues with which to judge the mode of reactions of the host against the transplant. In chickens, on the other hand, transplantation of the thyroid gland, on account of its situation

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These investigations were carried on with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

1. Loeb, Leo, and Addison, W. H. F.: Arch. f. Entwcklngsmechn. d. Organ. **32**:44, 1911.

2. Schultz, W.: Arch. f. Entwcklngsmechn. d. Organ. **35**:484, 1912; **36**:353, 1913.

3. Danforth, C. H., and Foster, F.: J. Exper. Zoöl. **29**:52 and 443, 1928.

far down in the neck adjoining the thorax, is much more difficult to accomplish without killing the animal; we chose, therefore, as tissues to be transplanted, pieces of skin in addition to the xiphoid cartilage and adjoining tissues. However, in using this technic, we introduced a number of variable factors which complicated the results and rendered their interpretation less certain. Even the fate of autotransplanted skin in chicken may vary considerably because the unavoidable transfer of feathers with the epidermis may injure the transplant to a different degree in different cases; furthermore, the results may vary in accordance with the thickness of the connective tissue on which the skin is situated, and there are other changes brought about by the way in which the surrounding host connective tissue may affect the ultimate shape of the grafted skin. In transplantation of mammalian skin a cyst is readily produced, lined on the inside by the transplanted epidermis and filled with masses of keratin. In the case of bird skin, a typical cyst occurs less readily; the skin is usually folded on itself without the typical formation of a cyst, although an imperfect cyst may form. In other cases, however, the epidermis may be everted so that the epidermis lines the outer instead of the inner side of this structure, and thus a nonspecific injury of the transplant tends to occur. Moreover, the epidermis as such, being a less sensitive reagent than the thyroid gland, is a less suitable indicator of the reaction of the host against the graft. As far as the xiphoid cartilage is concerned, the variety of associated tissues transplanted with it is usually less in birds than in mammals. In the majority of cases, this graft contains merely cartilage tissue with some adjoining dense tendon-like structures consisting of parallel strands of very dense fibrous tissue separated by rifts, in which the cells are situated.

There is a last difficulty which one has to face in such experiments in birds. Here the lymphocytes dominate in the circulation to a much greater extent than in mammals; the reaction of the host against the graft, a reaction in which, as we have shown previously, the lymphocytes play a dominating part, tends, therefore, to become very intense even when the discordances of either specific or nonspecific character are relatively slight, and this fact makes accurate grading of the degree of the reaction difficult. On the other hand, it is possible by means of transplantations in birds to study certain activities of lymphocytes more readily than by means of mammalian transplantations.

EXPERIMENTAL OBSERVATIONS

Series 1.—We first studied autotransplantation. Examination took place between five and thirty-five days after the transplantation of the tissue; altogether fifty-one pieces of tissue were studied microscopically. As early as from five to seven days after the transplantation of skin, giant cells were found around the keratin occasionally, and connective tissue surrounded either the epidermis or the keratin. The epidermis of the transplants showed variable degrees of preserva-

tion; it was preserved in some cases and partly or entirely destroyed in others. When the transplanted epidermis and cutis were destroyed, the growing connective tissue of the host moved toward the keratin, and here cells situated in the connective tissue formed epithelioid and giant cells around the keratin. A connective tissue capsule often encircled the keratin masses with the giant cells. In other cases the transplanted epidermis was well preserved and formed a cyst in the center of which was keratin. In the preserved epidermal cells mitoses occurred, and, as a rule, the epidermis was best preserved when it rested on the transplanted cutis. Either collections of lymphocytes were lacking, or very small clumps of lymphocytes arranged around vessels could be seen. They did not penetrate into the epidermis. If keratinized material had been carried into the connective tissue, a few lymphocytes collected around it. Cartilage and the surrounding tendon-like connective tissue were usually well preserved; but occasionally some small areas of necrosis occurred in either or both of them. Lymphocytes were lacking altogether, or only very small collections formed around particles of fat tissue transplanted with the cartilage or around some foreign body. These lymphocytes usually were arranged around vessels; they were apparently not attracted by the cartilage or tendon-like tissue, as such.

Sometimes there took place under these conditions a disequilibration between the cartilage and the connective tissue. Cells from the surrounding connective tissue or from the tendon-like tissue moved toward the piece of cartilage and collected around it. Connective tissue cells moved around the piece of cartilage in a concentric direction and formed a capsule about it. They often penetrated also into the periphery of the graft in a concentric direction corresponding to the fibrillar structure of the cartilage as indicated by the long axes of the cartilage cells. In some cases they then turned in a direction approximately at right angles to the long axis of the cartilage cells, thus penetrating slightly into the interior of the cartilage. Moreover, they possessed the power to split and dissolve the cartilage; in doing so, they sometimes enlarged. Either in the cartilage or in the surrounding dense fibrous tissue, some cells coming from the connective tissue could change into epithelioid or giant cells, especially in places where an obstacle presented itself to their progress. The connective tissue cells also accompanied some vessels which grew into the cartilage. It seemed that the connective tissue was especially attracted by large cell epiphyseal cartilage. No mitoses were observed either in the connective tissue or in the cartilage cells. In some instances, also, transplanted fat tissue and bone marrow with myelocytes were well preserved.

We found then, on the whole, that the transplanted cartilage was well preserved, and in no case did we find extensive collections of lymphocytes around it. On the other hand, active migration on the part of the connective tissue was often noticeable, and giant cells developed around hard and rough material.

Series 2, 3 and 4.—We shall now compare with the results of autotransplantations those obtained in a series of syngenesiotransplantations (series 2) in which pieces were transplanted from brother to brother (or sister); in some of these experiments strains of chickens that had been inbred for a number of years were used. Altogether seventy-nine pieces of skin and cartilage were transplanted, and the examination took place from five to thirty-five days after the transplantation. In series 3, the same kinds of tissues were transplanted into other, not near-related chickens belonging to the same variety, such as White Leghorn or Rhode Island Red chickens. As a rule, tissues were exchanged between birds belonging to two strains of the same variety obtained from different breeders. In some of these experiments long inbred strains were used. Here, twenty-nine transplants of skin and the same number of transplants of cartilage, totaling fifty-eight pieces, were

examined at periods varying between nine and thirty-five days following transplantation. In series 4, transplantations were carried out between birds belonging to different varieties, such as White Leghorn, Plymouth Rock and Rhode Island Red. Forty-six pieces of skin and fifty-one pieces of cartilage, making altogether ninety-seven pieces, were examined from eight to thirty-five days after transplantation. We shall not give a complete survey of our findings, but shall report on some observations made at certain periods following transplantation.

(*a*) From the Fifth to the Ninth Day Following Transplantation. Series 2: As early as after five days, small masses of lymphocytes were found around some capillaries. Occasionally collections of lymphocytes were somewhat denser. During the eighth and ninth days, lymphocytes moved into the fibrous tissue and began to dissolve it. They were found also in the fibrous tissue underneath the epidermis in the skin transplants. They penetrated into the tendon-like transplanted fibrous tissue, primarily along the preformed longitudinal interstices, which offered a path of least resistance. In some collections of lymphocytes small particles of detached fibrous tissue of the host were found, which we may take as an indication of the destructive action of the lymphocytes. Connective tissue cells, enlarged and with ameboid processes, invaded the peripheral parts of the cartilage and the necrotic bone. The transplants were surrounded by connective tissue capsules, which also sometimes contained a few strands of lymphocytes.

Series 3 and 4: The findings were similar; the lymphocytic infiltration on the whole was slight, although it was possibly a little more extensive and denser than in series 2.

Series 4: Some lymphocytes invaded the epidermis and the tendon-like fibrous tissue. Giant cells were found around the keratin, especially where the epidermis was missing. The latter seems to exert a certain protective effect against the invasion of the transplant by the connective tissue of the host.

(*b*) From the Tenth to the Eleventh Day Following Transplantation. Series 2: The lymphocytic infiltration on the whole was not very extensive, although in some places it may have been more marked; some lymphocytes penetrated into the transplanted epidermis.

Series 3: The tendon-like fibrous tissue was invaded by enormous masses of lymphocytes. The lymphocytes accumulated especially around blood vessels and united into nodes. They invaded the epidermis, a part of which they destroyed. Connective tissue cells, as well as nodes of lymphocytes, invaded also the tendon-like fibrous tissue, the lymphocytes as usual moving especially in the preformed longitudinal interstices; the lymphocytes entered also cartilage and bone and destroyed both tissues. Some giant cells formed around necrotic areas of cartilage. Although the lymphocytic infiltration was marked, it affected as yet mainly the connective tissue around the transplants and to a less extent the transplanted tissues themselves.

Series 4: A system of lymphocytic nodes had developed around vessels. The conditions here were similar to those seen in series 3.

(*c*) From the Twelfth to the Thirteenth Day Following Transplantation. Series 2: After thirteen days, we found for the first time in syngenesiotransplants dense masses of lymphocytes in the connective tissue surrounding the transplants, also nodes of lymphocytes directly around the cartilage, as well as tendon-like fibrous tissue around the epidermis. Within some of the lymphocytic nodes there were seen remnants of muscle fibers and detached pieces of fibrous tissue, which also may be taken as an indication of the destructive action of the lymphocytes; these cells also entered the epidermis and broke into the keratin. In the dense fibrous tissue they moved with ameboid processes in the direction of the fibers.

The transplanted muscle tissue was necrotic; the transplanted dense tendon-like fibrous tissue also contained some necrotic areas.

Series 3: Here, too, dense masses of lymphocytes, partly in the form of distinct nodes, surrounded the transplant including the cartilage. Lymphocytes penetrated also into living epidermis and injured it.

Series 4: Again, dense masses of lymphocytes were found, not only surrounding the cartilage, but penetrating into it and replacing a great part of this tissue. While the dense fibrous tissue inhibited and retarded to some extent the advance of the lymphocytic masses into the transplant, the lymphocytes succeeded in boring their way also into this fibrous tissue, and in the end these cells in compact masses invaded and then dissolved it, even if its cells were still alive. Lymphocytes and also connective tissue cells, in their destructive action, detached pieces of cartilage and bone from the transplant. In the cartilage, the lymphocytes opened the spaces in which the cartilage cells were lying and filled these cavities. Also around the skin transplant there were in places very dense infiltrations resembling lymph nodes; yet in the transplanted epidermis mitoses could still be seen, and great accumulations of lymphocytes separated the epidermis from the surrounding connective tissue. We then noticed for the first time collections of lymphoblasts in the peripheral parts of these nodes. The lymphocytes as a rule accumulated first around blood vessels. While in the majority of these specimens there was thus a very marked lymphocytic infiltration, it was not present in all transplants, some variations occurring in this respect.

(*d*) From the Twenty-Fifth to the Twenty-Eighth Day Following Transplantation. Series 2: Cartilage and tendon-like fibrous tissue were found to a variable degree dissolved and replaced by nodes of lymphocytes, the peripheries of which were in certain cases surrounded by lymphoblasts; in other instances lymphoblasts themselves formed follicles situated in the peripheries of the lymphocytic infiltrations. Mitoses occurred in these lymphoblasts; occasionally hemorrhages were seen in the nodules. Also lymphoblasts were able to invade and destroy the tendon-like tissue. Lymphocytic nodes surrounded the keratin of the skin transplants. Connective tissue in places grew into necrotic areas of cartilage, leading to the development of fibrillar connective tissue.

Series 3: Necrotic tissue, cartilage and tendon-like connective tissue were surrounded by a number of lymphocytic nodes; from these nodes lymphocytes penetrated into the cartilage, which they dissolved, and, furthermore, into the tendon-like tissue in the direction of the preformed interstices. Likewise connective tissue cells penetrated into the cartilage and also into bone and dissolved these tissues.

Series 4: Enormous masses of lymphocytes were found around the various tissues of the transplants. They formed a continuous ring of lymphocytic nodes about these structures, penetrating into them in strands and, in the end, overwhelming and destroying them. The cartilage was also invaded by these cells, preferably in the direction of the fibrillae in the cartilage; it was evidently easier for them to proceed in these interstices than to push their way into the cartilage at right angles to this direction. They were furthermore able to convert the tendon-like tissue into a finely fibrillar material which stained much more lightly with eosin than the original tissue which they had invaded. Some lymphocytes enlarged while entering the cartilage. In certain instances bone also was overwhelmed with lymphocytes, and lymphocytes were seen between the keratin material, which, in addition, was surrounded by hyaline masses corresponding to necrotic giant cells. Connective tissue cells likewise invaded the cartilage and here assumed the character of a myxoid connective tissue.

COMMENT AND CONCLUSIONS

From these experiments we may draw the following conclusions: In birds, as well as in other classes of vertebrates, the relation of the organismal (individuality, race) differentials is the essential factor which determines whether or not the host will affect the transplant injuriously. In autotransplantations injurious reactions are lacking, except those which are due to extraneous accidental factors, while in all other transplantations (syngenesiotransplantations, homeotransplantations and interracial transplantations) they are present. They were also found in former experiments in which homeotransplantation and heterotransplantation of skin was carried out in birds.

In birds the lymphocytes represent a much higher percentage of the leukocytes circulating in the blood than in mammals; correspondingly in birds the lymphocytic reaction against a transplant is much more intense than in mammals if the organismal differentials of the host and the transplant differ. Thus even after syngenesiotransplantation in inbred strains of fowls the lymphocytic reaction may be very intense. This great intensity of reaction and the great sensibility of birds to strange transplants, resulting from the great activity of the lymphocytes, make difficult a finer gradation of the intensity of the reaction in accordance with the relationship between the host and the transplant, as difficult as would be an attempt to measure differences in weight as great as many grams by using a balance responding to differences of a fraction of a milligram. Nevertheless, there are indications that, on the average, the reactions were least intense in transplantations between brothers belonging to inbred strains and most intense in cases of interracial transplantation. However, since in the former type in individual instances there occur certain variations in the strength of reaction, which sometimes can be very strong, it would be difficult to prove such a gradation in an exact manner, although there is, as stated, an indication that a relation between the intensity of reaction and the distance of relationship between the organismal differentials exists.

Because of the great intensity of the lymphocytic reaction in birds against transplants of chicken tissues, especially those of cartilage and tendon-like fibrous tissue and even bone, it is possible to recognize the marked ability of lymphocytes to invade, destroy and, in particular, to dissolve resistant tissues like cartilage and dense fibrous tissue. In homeotransplantations and interracial transplantations (series 3 and 4) extensive lymphocytic infiltration was first observed after ten and eleven days, while in syngenesiotransplantation (series 2) it was first observed after thirteen days. The lymphocytes either dissolve such grafts or leave merely a finely fibrillar material; in some cases their destructive action may detach pieces of fibrous and of muscle tissue, which are then

included in nodular accumulations of these cells. The lymphocytes invade the transplant preferably in the direction of least resistance; thus the pseudopods, which the lymphocytes as well as connective tissue cells send out while migrating, have evidently the best chance of effecting locomotion of the whole cell. However, lymphocytes are able to penetrate also at right angles to these preformed fissures and to invade the tissue directly. The same observation can be made in the case of connective tissue cells; but it seems that while, as a rule, connective tissue cells invade only dead tissue, lymphocytes more readily invade and destroy living structures. However, connective tissue cells are able to push their way between living structures also and thus to separate them.

The reaction of lymphocytes against transplants leads to the accumulation of these cells around blood vessels. Here they form follicle-like masses; but toward the end of the second week or during the third week there appear, in addition to the small lymphocytes, collections of large lymphoblast-like cells, in which mitoses are frequent. These cells may collect also in the form of small follicles, situated in a diffuse mass of lymphocytes, especially in the periphery of the connective tissue and surrounding the transplants, and they may also invade and destroy the strange tissue. Such lymphoblastic accumulations remained visible until the end of the period during which we followed the fate of the transplants; they were found in syngenesiotransplantations as well as in homeotransplantations and interracial transplantations, but around interracial transplants collections of lymphoblast-like cells appeared first in the peripheral parts of the lymphocytic nodes, about thirteen days after transplantation, while lymphoblasts with mitoses appeared in the second series at sixteen days.

We may therefore conclude that birds react quite generally in a very intense manner against the presence of tissue possessing strange organismal differentials. Danforth and Foster [3] apparently arrived at a different conclusion. They transplanted skin flaps from recently hatched chicks either to other chicks of the same inbred races or to other races, such as White Leghorn and Plymouth Rock. In many cases the pieces of skin healed in permanently in chickens belonging to other races, although the best results were obtained in interchange of skin between members of the same inbred race; but this may have been due to accidental factors rather than to a similarity of the organismal differentials between the host and the transplant. These authors stated their belief that individuality differentials exist in birds in isolated instances.

However, this interpretation of their results is not the only one possible. In the first place, we must consider that they used for their transplantations recently hatched chicks rather than adult birds; and judging from experiences with mammals and also with birds, we should expect the reactions in very young hosts to be much milder. It is furthermore

possible that when skin grafts heal in, in very young hosts, a gradual adaptation may take place between the host and the transplant so that the grafts remain attached to the host even after the animal has reached the adult stage. Secondly, the "take" or "nontake" of a transplant is not a fully satisfactory test for the degree of incompatibility of two organismal differentials. A much finer index consists in the measurement of the intensity of the reaction of the lymphocytes, connective tissue and blood vessels of the host against the transplant. Using such tests, we were able in these experiments to prove that the individuality differentials even between brothers belonging to inbred strains may differ from each other, and that homeotransplantation and interracial transplantation lead to very severe reactions of the host against the transplant. Our results also accord with the findings of May,[4] who observed that in adult lizards homeotransplantation of skin does not succeed, whereas autotransplantation is successful.

SUMMARY

Organismal and, in particular, individuality differentials are present in birds, as well as in mammals; this is proved by the difference in the lymphocytic reactions after autotransplantation and after syngenesiotransplantation and homeotransplantation. However, even relatively small differences in the constitution of the organismal differentials cause so marked a lymphocytic reaction in birds that quantitative determinations of differences in the organismal differentials between different individuals in this species are rendered difficult.

In birds the activity of lymphocytes and lymphoblasts is so intense in transplantations other than autotransplantation that the destructive effect, which both small and large lymphocytes exhibit, can be readily recognized. This destructive effect applies even to cartilage and bone substance.

4. May, R. M.: Brit. J. Exper. Biol. **1**:539, 1924; Transplantation animale, Paris, Gauthier-Villars, 1932.

SERPENTINE ANEURYSM OF THE INTERNAL CAROTID ARTERY

WITH RESULTING ENCEPHALOMALACIA AND CEREBRAL HEMORRHAGE

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In a previous communication [1] anomalies of the circle of Willis were discussed which were thought to play an important rôle in the causation of encephalomalacia and cerebral hemorrhage. It was pointed out that there is an increasing tendency to attribute to functional abnormalities some anatomic changes which are undoubtedly the result of vascular lesions. This is particularly true in certain instances of encephalomalacia and cerebral hemorrhage. For this reason, as was stated before, every demonstrable morphologic cause of vascular disturbance should be searched for, carefully evaluated and ruled out before an explanation is given based on functional disturbance. Anomalies of the circle of Willis with resulting interruption of the circulation between the internal carotid and the vertebral arteries may, under certain circumstances, form the anatomic basis for encephalomalacia and cerebral hemorrhage.

The purpose in this communication is to describe three instances of encephalomalacia and cerebral hemorrhage, the causes of which could not be determined until the internal carotid arteries were completely dissected and severe occluding lesions found in them. These lesions were thought to have been the etiologic factor in the lesions of the brain.

REPORT OF CASES

CASE 1.—A 75 year old woman who had shown moderate senile dementia for the previous eleven years was found unconscious at home the day she was admitted to the hospital. On examination there were Cheyne-Stokes breathing and right-sided hemiplegia. The pupils were contracted and did not react to light. There were moist râles on the right side of the chest; the heart tones were faint. The face and the lower extrèmities were edematous. At the time of her admission to the hospital the rectal temperature was normal, but in the next five hours it rose to 101.6 F. The white blood cell count was 24,700, with 90 per cent polymorphonuclear leukocytes. The arterial blood pressure was 144 systolic and 80 diastolic. The clinical impression was that she had suffered a cerebral hemorrhage of the left hemisphere involving the internal capsule. The eyegrounds were not examined.

From the Department of Pathology of the Nelson Morris Institute, Michael Reese Hospital.

1. Saphir, O.: Anomalies of the Circle of Willis with Resulting Encephalomalacia and Cerebral Hemorrhage, Am. J. Path., to be published.

At autopsy, the heart weighed 325 Gm. There were coronary arteriosclerosis, myocardial fibrosis and chronic passive hyperemia of the various organs. There was a generalized arteriosclerosis, with marked thickening of the arteries at the base of the brain. No occlusion of the lumens of these vessels could be demonstrated. There were nephrosclerosis of the arterial variety and bilateral confluent bronchopneumonia. The skull revealed no evidence of fracture. There was a cerebral hemorrhage involving the left capsula extrema, claustrum, external capsule and putamen and the posterior region of the left internal capsule. The adjacent white substance of the brain up to the anterior portion of the left parietal lobe was grayish pink and finely granular and did not reveal the normal architecture. The left lateral ventricle was filled with clotted blood.

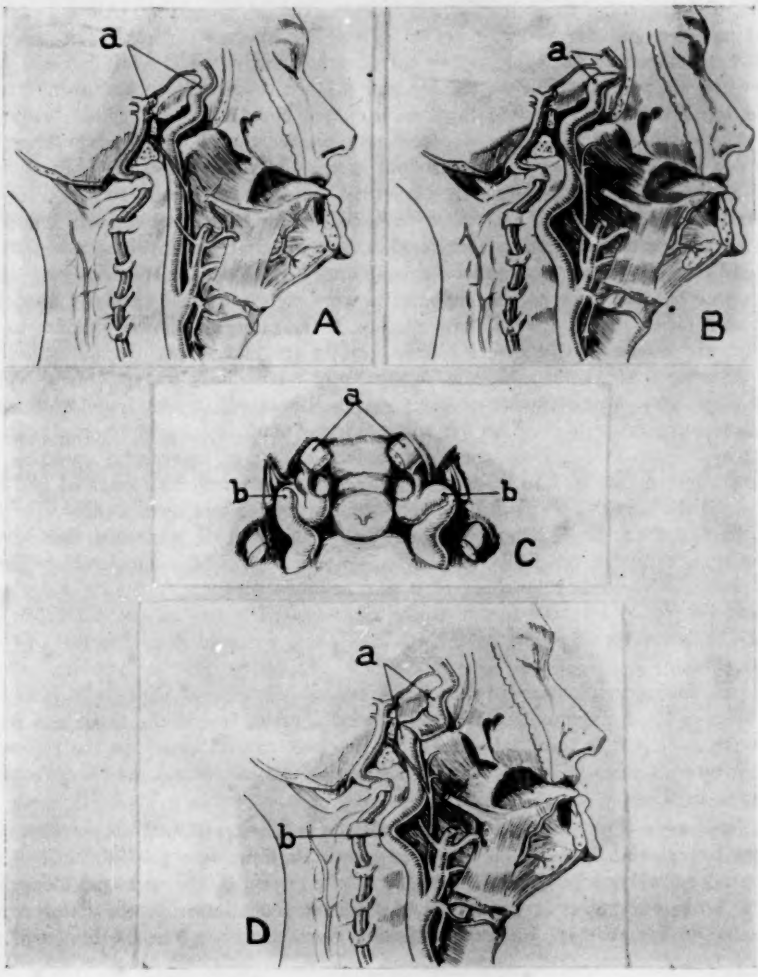
The cerebral arteries were dissected, but neither they nor their finer branches showed occluding lesions. The vertebral arteries were dissected next but showed no occluding lesion, though arteriosclerotic plaques were numerous. Since no definite explanation of the cerebral hemorrhages and encephalomalacia could be given, the possibility that a functional abnormality in the involved vessels had caused these conditions was seriously considered. However, before this explanation was accepted, both internal carotid arteries were completely and carefully dissected. These arteries, in the posterolateral regions of the pharynx, showed distinct tortuosity; the lumens of the arteries were slightly encroached on. In the region of the cavernous sinus, on each side, a firm, tumor-like mass was palpated. When the structures of the cavernous sinuses were dissected, it was found that what had seemed, on palpation, to be tumors were markedly rigid internal carotid arteries. Each vessel, from the intracranial opening of the carotid canal to the point where it divides into its final ramifications, was markedly elongated and had assumed the form of an S with very sharp curves. The curves closely approximated one another. When the left artery was opened, it was seen that at the middle curvature of the S-like loop a ridge was formed which completely occluded the lumen. The ridge consisted of a thin calcified plaque. A similar ridge was found projecting into the lumen of the right carotid artery at the corresponding site. This artery, however, though its lumen was extensively encroached on, was still patent.

Histologic examination of the brain revealed in many fields only indistinct outlines of brain tissue with many red blood corpuscles free in the tissue and many scavenger cells. Sections taken from the internal carotid artery in the region of the cavernous sinus showed areas of hyalinization and calcification and large atheromatous cavities.

Summary.—The brain of a 75 year old patient who was found unconscious with right hemiplegia showed large areas of hemorrhage and encephalomalacia. Each internal carotid artery showed tortuosity in the region of the neck and elongation with S-like curvatures in the region of the cavernous sinus. In the latter region the left artery revealed complete occlusion of its lumen, while the lumen of the right artery was markedly narrowed.

Case 2.—A white woman, 60 years of age, who had had diabetes mellitus for a number of years, was admitted to the hospital because of pains in and discoloration of the right leg and toes. On physical examination the heart revealed a systolic murmur at the apex and at the base. The arterial blood pressure was 170 systolic and 62 diastolic. The plantar surface of the left foot revealed early gangrene. The urine contained dextrose (4+), acetone (4+) and diacetic acid (3+), and the blood contained 162 mg. of dextrose per hundred cubic centimeters. Five days before death, right-sided hemiplegia without coma and motor aphasia developed. The patient finally contracted bronchopneumonia, from which she died.

Autopsy revealed bilateral bronchopneumonia, generalized arteriosclerosis, coronary arteriosclerosis, myocardial fibrosis and chronic passive hyperemia of the various organs. Medial calcification of the femoral, popliteal and anterior and posterior tibial arteries was found, with occlusion of the lumens in various places. The left foot and the toes of the right foot were gangrenous. The brain revealed



Serpentine aneurysms of the internal carotid artery: *A*, the course of the carotid artery; the petrous portion of the artery is shown at *a*. *B*, carotid artery in case 1, with a serpentine aneurysm in the region of the cavernous sinus, shown at *a*. *C*, optic nerves (*a*) and internal carotid arteries in the region of the cavernous sinus in case 1, seen from above (*b*). *D*, carotid artery in case 3, with a serpentine aneurysm in its petrous portion (*a*) and a tortuosity in the posterolateral region of the pharynx (*b*).

a large area of softening, dark gray and finely granular, which extended through the white substance of the left temporal lobe to involve portions of the left parietal and the adjacent portion of the occipital lobe. The arteries at the base of the brain showed marked arteriosclerosis. Each internal carotid artery, in the region of the cavernous sinus, was lengthened and revealed an S-like tortuosity. The lumen of the left internal carotid artery corresponding to the distal curvature of the S loop was occluded by a ridge which was formed by a portion of the inverted wall of the artery. The lumen of the right carotid artery was markedly narrowed. The branches of both internal carotid arteries were carefully dissected, and no occluding lesions were demonstrable.

Histologic examination of the brain revealed its architecture obscured in many fields. There was an extensive infiltration by scavenger cells, and in many sections these cells were seen surrounding small blood vessels. A few lymphocytes were also present. Other sections showed many red blood cells free in the tissue. Sections through the carotid artery revealed areas of hyalinization and calcification and atheromatous cavities.

Summary.—The brain of a 60 year old woman in whom hemiplegia developed revealed encephalomalacia with areas of hemorrhage. On each side the internal carotid artery was markedly lengthened and tortuous in the region of the cavernous sinus. The lumen of this portion of the left artery was occluded.

CASE 3.—A 70 year old woman suffered severe dizziness and gradually sank into coma on the morning of her admission to the hospital. She had been well prior to this, and her arterial blood pressure had never been high. On examination it was noted that the pupils did not react to light and were slightly irregular. No abnormalities of the heart were found. The pulse rate was 68 beats per minute, and the arterial blood pressure was 148 systolic and 80 diastolic. Coarse râles were heard in both lungs. The Babinski reflex was positive bilaterally. The eyegrounds could not be examined.

Autopsy revealed generalized arteriosclerosis with coronary arteriosclerosis, myocardial fibrosis and chronic passive hyperemia of the various organs. There were acute purulent bronchitis and bilateral bronchopneumonia. The skull presented no abnormalities. The subarachnoid space contained a moderate amount of hemorrhagic liquid. A large area of hemorrhage and encephalomalacia was found in the right frontal lobe and in the white substance of the right parietal lobe. The hemorrhage extended into the right lateral and third ventricles. The right capsula extrema, external capsule, claustrum and lenticulate nucleus were almost completely destroyed. The right internal capsule, however, was well preserved with the exception of its most lateral portion. The arteries at the base of the brain showed severe arteriosclerotic changes. Each internal carotid artery was carefully dissected throughout its whole course. There were distinct tortuosity and lengthening in the posterolateral regions close to the pharynx. The lumens in these regions were slightly narrowed. The most severe changes were found in the petrosal portion of each internal carotid artery, within the carotid canal. Here the arteries were almost completely transformed into pipelike structures. Both arteries were curved into S-like loops with production of ridges. That in the right artery had led to occlusion of the lumen. In the region of the cavernous sinus each artery was thickened but not elongated.

Histologic examination of the brain revealed large areas of hemorrhage with only remnants of brain tissue. In other areas the architecture of the brain could not be made out, the nuclei were poorly stained, and a few scavenger cells were observed.

Summary.—The brain of a 70 year old woman who died in coma revealed hemorrhages and encephalomalacia. The internal carotid arteries in their cervical portions showed distinct tortuosity. In their petrosal portions these arteries were lengthened and tortuous, forming S-like curves with production of ridges, which caused marked narrowing of the lumen of the left, and occlusion of the lumen of the right, artery.

COMMENT

These three instances are significant in that they reveal severe occluding lesions in the internal carotid arteries at locations where such lesions as a rule are not looked for, namely, in the region of the cavernous sinus and within the carotid canal of the temporal bone. Narrowings of the lumens were also present in the first portions of the internal carotid arteries, in the pharyngeal region. The encroachment on the lumens was the result of the tortuosity of the vessels with inversion of parts of their walls and formation of bends or kinks.

The tortuosity of the arteries may be referred to as cirsoid aneurysm, following the classification of Kaufmann [2] and of Karsner.[3] Kaufmann stated that in instances of cirsoid aneurysm the involved artery is dilated, elongated and tortuous. Karsner emphasized that the cirsoid aneurysm is distinctly more common in the smaller vessels, such as the temporal arteries, where there is general dilatation associated with lengthening and tortuosity. In this form of aneurysm the primary condition is some variety of arteriosclerosis. Jores,[4] on the other hand, stated that the dilatation of the lumen of an artery combined with tortuosity should be referred to as serpentine aneurysm. He reserved the term "cirsoid aneurysm" for only those instances in which branches of the primarily involved artery are similarly dilated and tortuous. If Jores' classification is used, the lesions described here should be classified as serpentine aneurysms.

Tortuosity of the internal carotid artery in the posterolateral region of the pharynx is well described by Brown-Kelly,[5] who mentioned all degrees of bending between extreme tortuosity and slight curvatures. Skillern [6] described a marked degree of tortuosity of the artery in the cadaver of a person apparently 60 years old. The artery was uniform in caliber. Beginning at the skull and extending two thirds of the distance to

2. Kaufmann, E.: Lehrbuch der speziellen pathologischen Anatomie für Studierende und Aerzte, ed. 7 and 8, Berlin, W. de Gruyter & Co., 1922.

3. Karsner, H.: Human Pathology, Philadelphia, J. B. Lippincott Company, 1926.

4. Jores, L., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1924, vol. 2, p. 608.

5. Brown-Kelly, A.: J. Laryng. & Otol. **40**:15, 1925.

6. Skillern, P. G.: J. A. M. A. **60**:172, 1913.

the bifurcation of the common carotid artery, an S-shaped tortuosity was observed, the bends of which extended at no time more than 1 cm. from a line representing the axis of the normal artery. Fisher [7] stated that such tortuosity may be explained phylogenetically, since in many of the mammalia the internal carotid artery is even more tortuous than it is in man, and quoting Chauveau he stated that the internal carotid artery of the seal, for instance, is actually forty times as long as the distance it has to traverse. This, however, could not be confirmed by Carmel.[8] Brown-Kelly described the tortuosity of the artery in persons of varying ages and stressed its relation to senile (arteriosclerotic) changes and to congenital malformations. Streit [9] stated that arteriosclerosis was the principal cause of the tortuosity which he described in the internal carotid artery. Schaeffer [10] reviewed the literature and stressed the fact that age has no bearing on the development and presence of tortuosity in an internal carotid artery. Carmack [11] stated that aberrations of the internal carotid arteries probably occur more frequently than is realized, and that their occurrence invariably brings the vessel closer to the tonsil and pharynx.

Jastram,[12] quoting Broesike, named five locations where curvatures of the internal carotid artery may predispose to tortuosities: (1) just behind the bifurcation; (2) just beneath the base of the skull; (3) within the carotid canal; (4) within the sulcus caroticus close to the sella turcica; (5) in the region of the impressio carotica of the sphenoid bone. It may be mentioned that no reference to tortuosity of an internal carotid artery within the carotid canal and cavernous sinus could be found in the literature studied.

It seems clear that in those regions where the internal carotid artery normally is slightly tortuous, this tortuosity may increase in arteriosclerosis. Jores called attention to the fact that the arteriosclerotic process is often characterized by dilatation of the involved blood vessels combined with tortousity. In none of the three instances described, however, was the tortuosity of the internal carotid artery in the posterolateral region of the pharynx severe enough to have led to a marked narrowing or occlusion of its lumen.

The sigmoid tortuosity of the artery within the carotid canal of the temporal bone was much more significant, because it had led to the formation of a ridge which occluded the lumen. The space within the

7. Fisher, A. G. T.: Lancet **2**:128, 1915.
8. Carmel, A. G.: Anat. Rec. **39**:343, 1928.
9. Streit, H.: Ztschr. f. Hals-, Nasen- u. Ohrenh. **30**:315, 1931.
10. Schaeffer, J. P.: J. A. M. A. **77**:14, 1921.
11. Carmack, J. W.: Laryngoscope **39**:707, 1929.
12. Jastram, M.: Beitr. z. klin. Chir. **93**:341, 1914.

carotid canal is limited. The walls of the canal, being osseous, cannot expand when the artery, as a result of arteriosclerosis, tends to become wider and elongated. The inevitable result must be inversion of parts of the wall with formation of kinks and, because of the elongation, a change from the normal simple curve in this location to the S-like tortuosity. The walls of the carotid canal were still smooth. This indicates that the changes were not old enough to have produced erosions of the adjacent bony structures.

In the first two instances the occluding lesions of the internal carotid arteries were found in the region of the cavernous sinus. The sigmoid tortuosity in the first skull was so marked that it gave, on palpation, the impression of a tumor. And yet, grossly, little was seen until the dura was removed and the cavernous sinus was dissected. The increase in length of the carotid artery from where it entered the cranial cavity to the point at which it divided into its final branches was about three times the normal length in this area in the first instance, and about two and a half times the normal length in the second instance. The disproportion between the limited space in this region and the increase in length had led to the sigmoid tortuosity of the artery. The combined effect of the tortuosity and the widening of the lumen because of the arteriosclerosis had caused inversion of the wall at a point of the S-like curvature with formation of a ridge which resulted in the occlusion of the lumen.

As has been shown, an occluding lesion in the internal carotid artery was present in every instance. This lesion must be considered as at least one factor in the causation of the encephalomalacia and cerebral hemorrhages. The other factors were the arteriosclerosis of the arteries at the base of the brain and the coronary arteriosclerosis with resulting myocardial fibrosis which was found in every heart.

Salinger and Pearlman [13] recently discussed ligation of the internal (common) carotid artery. They stressed the fact that in a number of patients one internal carotid artery can be ligated without causing vascular disturbances of the brain, because of the adequate supply through the circle of Willis. In senescence, however, with increasing arteriosclerosis of the arteries at the base of the brain and consequent narrowing of the lumens of the arteries it seems clear that the anastomoses cannot function as well as normally. And yet the circulation of the brain may be adequate to prevent vascular disturbances even though one internal carotid artery is occluded. In patients who have coronary arteriosclerosis with increasing myocardial fibrosis, however, the point finally will be reached at which the myocardium becomes insufficient, either gradually or suddenly. Hence, if the vis a tergo is reduced, there may occur vascular insults to the brains of those patients

13. Salinger, S., and Pearlman, S. J.: Arch. Otolaryng. **19**:464, 1933.

who show lesions in the circle of Willis or in the internal carotid arteries or in both. It seems important to consider these three factors in every instance of encephalomalacia and cerebral hemorrhage in the absence of thrombi or emboli in the arteries of the brain.

The question arises why the entire hemisphere of the brain was not involved when one internal carotid artery was occluded and there was a considerable amount of arteriosclerosis in the vessels at the base of the brain. Because the vessels of the circle of Willis and the vertebral arteries were patent, there evidently was sufficient collateral circulation to prevent encephalomalacia being caused by the vascular changes as long as the heart maintained its normal function. When the myocardium began to fail the collateral circulation was apparently insufficient, and encephalomalacia and hemorrhage followed.

In evaluating the causes of vascular disturbances of the brain, the circle of Willis must be given careful consideration, as was brought out in the previous communication.[1] It was shown that anomalies of the circle of Willis exist which result in complete separation of the two main arterial channels of the brain. Such anomalies are encountered not infrequently in routine autopsies and per se are of no consequence. Two brains were described [1] which revealed areas of encephalomalacia and cerebral hemorrhage without occluding lesions in the supplying arteries. In both instances, however, the blood could not pass through the posterior communicating arteries, and there was also evidence of myocardial failure. In these two instances, one of the three factors mentioned was very pronounced, namely, the absence of the collateral supply through the circle of Willis. In the three cases reported here, another of the three factors occupied the paramount place, namely, arteriosclerosis of the internal carotid artery with occlusion of its lumen. As mentioned before, in every instance of this series evidence of myocardial failure was also found. This was based, first, on the myocardial fibrosis and, second, on the passive hyperemia of various organs. It may be especially emphasized that this conception of the pathogenesis of encephalomalacia and cerebral hemorrhage is based on morphologically demonstrable lesions and deductions drawn from these findings.

In this study I have attempted to show the importance of post-mortem examination of the entire body in the explanation of these brain changes, and particularly examination of the heart and internal carotid arteries, as well as of the brain. I have also attempted to show the importance of the examination of the various organs for evidence of chronic passive hyperemia. In every instance of vascular disturbance of the brain the entire course of the internal carotid arteries should be examined in order to locate morphologically demonstrable causes of

the brain lesions. An examination of both vertebral arteries may also prove to be of value. All these factors should be looked for and carefully evaluated or eliminated before the pathologist resorts to an explanation primarily based on functional disturbances.

The eternal reproach with which the morphologist is confronted culminates in the accusation that his reasonings are "post hoc." Naturally, it is the duty of the pathologist to combine the various observations at autopsy and to try to relate the changes to primary lesions. In trying to adhere to this principle, occasional misconceptions may occur and a cause and effect relationship may be attributed to lesions which are only coincidental. However, it seems preferable for the morphologist to err in this respect rather than to resort too quickly to explanations which are not based on anatomically demonstrable lesions. It is conceivable that in the instances reported here and in the previously reported instances in which brains revealed anomalies of the circle of Willis, spasms of the arteries of the brain could have been held responsible for the brain changes, had the entire internal carotid arteries not been examined and had not sufficient attention been given to the circle of Willis. But since these lesions were found in addition to evidence of myocardial failure it seems clear that they can easily be correlated with the encephalomalacia and cerebral hemorrhages in a cause and effect relation. Perhaps more detailed postmortem examinations will result in the finding of anatomic lesions similar in some respects to those described here that will explain anatomic changes elsewhere which now are attributed to functional disorders.

SUMMARY

In every instance of encephalomalacia and cerebral hemorrhage, the internal carotid and vertebral arteries should be carefully examined throughout their course in order to locate morphologically demonstrable causes of the lesions of the brain. Three procedures should be observed in the explanation of these lesions in the absence of occlusions of the vessels at the base of the brain or of their branches: 1. The internal carotid and vertebral arteries should be examined throughout their course for occluding lesions. 2. The state of the circle of Willis should be studied with particular reference to congenital anomalies which may have caused an interruption of its continuity. 3. Morphologic evidence of cardiac failure should be looked for, such as coronary arteriosclerosis with resultant myocardial fibrosis and chronic passive hyperemia of the various organs. All these anatomic lesions should be searched for and carefully evaluated or eliminated before the pathologist resorts to an explanation based primarily on functional disturbances. Three instances of encephalomalacia and

cerebral hemorrhage are reported. Occlusions of the internal carotid arteries were found at locations where such lesions as a rule are not looked for, namely, in the region of the cavernous sinus and within the carotid canal of the temporal bone. The occlusions were the result of the tortuosity of the arteriosclerotic vessels with inversion of the walls and formation of bends or kinks. If Jores' classification is used, such a lesion of the artery is referred to as serpentine aneurysm.

STUDIES ON SPONTANEOUS RECOVERY FROM PNEUMOCOCCIC INFECTION IN THE GUINEA-PIG

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The studies here reported relate to two questions: 1. What interpretation in relation to resistance to infection may be placed on the local cellular reaction in the course of infection? 2. What significance in relation to infection may be placed on the phagocytic activity of leukocytes in the course of infection?

Since we wished to observe the differences occurring when infected animals die and when they recover spontaneously, it was necessary to permit the experimental infection to run its natural course; it was therefore possible to observe only a limited part of the reactions occurring in the infected animals.

MATERIAL AND METHODS

In all experiments guinea-pigs were used; in practically all cases a strain of Pneumococcus type I was used. The virulence of this strain was maintained by repeated passages through mice so that it was lethal for mice in dilutions of 10^{-8} or 10^{-9}. The guinea-pigs were infected by intraperitoneal injections of eighteen to twenty-four hour serum dextrose broth cultures; usually 0.25 cc. of an undiluted culture was administered, as it was found that a limited number of animals so infected survived. After infection, fluid was withdrawn from the peritoneal cavity at periods of thirty minutes, one hour, six or eight hours, twenty-four hours, two days and subsequently at twenty-four hour intervals; smears of the peritoneal exudate were made and stained with Wilson's stain (for counting cells and determining phagocytosis), and cultures of the fluid were made to determine the presence of the infecting organism. In a number of cases comparison was made of the peritoneal fluid studied by the stained smear with that studied by intravital staining; in practically all cases the percentage of mononuclears was higher in the intravitally stained specimens, so that there were from 5 to 10 per cent more mononuclears counted in these. Since there was this relatively small but fairly constant difference we retained the stained smear in counting cells. At least 200 cells were counted and in many cases from 500 to 900 cells; several portions of the smear were consistently examined. The phagocytosis was determined on the basis of the number of cells of each type containing bacteria and is therefore expressed as a percentage of phagocytosing cells. In addition, in a considerable number of cases, but not in all, blood cultures were taken during the course of infection.

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PNEUMOCOCCIC INFECTION IN THE GUINEA-PIG

Usually within thirty minutes and always within an hour after injection, the organisms had passed into the blood stream. The animals showed little evidence of illness within the first twelve hours, but by twenty-four hours they were listless, their hair was ruffled, and they ate and drank little. If they survived the first twenty-four or forty-eight hours they showed emaciation. The duration of the infection varied widely. Some animals (eleven) died within the first twenty-four hours; a considerable number (ten) survived for two, three or four days and a few (ten) for from five to ten days, only to die after these longer periods. A few (three) died after the tenth day. Eighteen survived the infection.

In all cases in which the survival was shorter than six or seven days the pneumococci were constantly demonstrable in the peritoneal fluid. When, however, the period of survival was longer than seven days, it often happened that a positive culture was not obtained from the peritoneal fluid for a day or for several days before death. In some cases even in the last culture taken before the death of the animal no pneumococci were cultivated; in other cases, however, the pneumococci, after being absent from cultures for a time, were demonstrable before the death of the animal. In those experiments in which the animals receiving injections survived, the organisms had usually disappeared from the cultures of the peritoneal fluid before or at about the fifth or sixth day and did not reappear. Since some animals died from twenty to thirty days after the injection of pneumococci in spite of the fact that the cultures of the peritoneal fluid had been negative for two days or more, it was essential to formulate criteria of recovery. The guinea-pigs which died never recovered from the emaciation which occurred during the acute stage of infection. Therefore, only animals which survived beyond thirty days after the injection and which regained their weight after the acute stage of the infection were classed as recovering animals.

THE CELLS IN THE NORMAL PERITONEAL FLUID

The cells normally present in the peritoneal cavity of the guinea-pig were studied by Gardner [1] with intravital stains. Our studies were, as stated, carried out by means of stained slides. In counts in twenty-four normal animals we found the percental distribution of the leukocytes to be: neutrophils, from none to 5; eosinophils, from 15 to 27; lymphocytes, from 8 to 19, and mononuclears, from 60 to 75.

CHANGES IN THE CELLULAR ELEMENTS OF THE PERITONEAL FLUID UNDER THE INFLUENCE OF IRRITANTS

Effect of Nonliving Irritants.—If any foreign material or other irritant is introduced into the peritoneal cavity (0.9 per cent sodium chloride solution, broth, aleuronat, silver nitrate solution), in the first six hours there will be a drop in the number of mononuclears, a relative rise in the number of lymphocytes and eosinophils and somewhat later a further fall in the number of mononuclears, a fall in that of lymphocytes and of eosinophils and an increase in number or an appearance of neutrophils. This early sequence of changes is constant and occurs as a response to the introduction into the peritoneal cavity of any irritant (including pathogenic bacteria).

Often at six hours the neutrophils have reached a maximum percentage varying between 58 and 90, and by twenty-four hours the percentage of these cells has

1. Gardner, L. U.: Proc. Soc. Exper. Biol. & Med. **26**:690, 1928-1929.

usually but not always begun to fall. The percentage of mononuclears is usually at the lowest at six hours and has begun to rise at twenty-four hours. Lymphocytes and eosinophils are frequently absent at six and at twenty-four hours, or may be present only in very small numbers. At forty-eight hours the neutrophils are still present, their percentage varying between 1 and 20; the percentage of mononuclears is approximately normal, and a few eosinophils and lymphocytes are present. It is, however, questionable whether we may state that the cellular contents of the peritoneal cavity have returned to normal, since neutrophils may persist (from 1 to 10) for from four to six days after the injection, and the eosinophils may remain relatively low for six days or longer. The complete restoration to normal is, therefore, a relatively slow process.

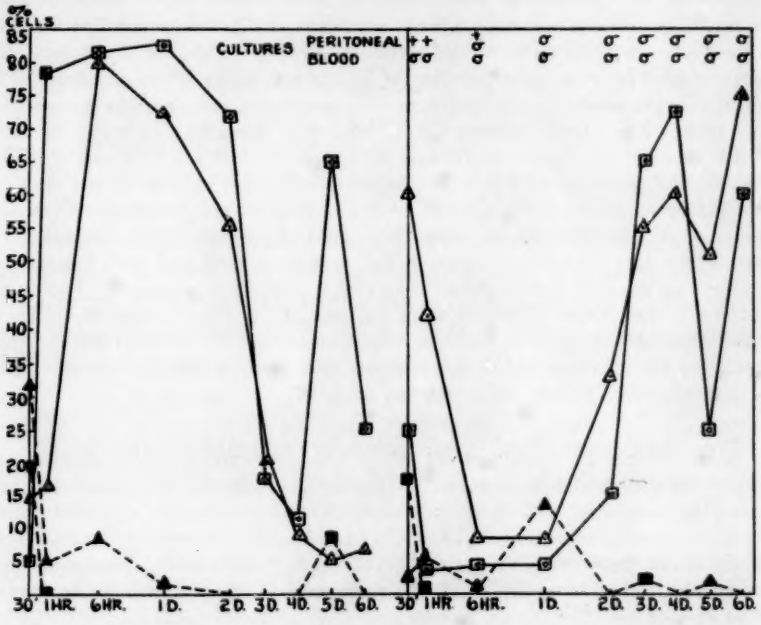

Chart 1.—Percentages of neutrophils, mononuclears and phagocytosing cells in peritoneal fluid of animals given intraperitoneal injections of avirulent (rough) pneumococci. Solid lines represent neutrophils (left side of chart) or mononuclears (right side); beaded lines represent phagocytosing cells.

Effect of Nonpathogenic Bacteria.—When avirulent or nonpathogenic bacteria were injected (2 cc. of a twenty-four hour broth culture of an avirulent strain of hemolytic streptococci, Corynebacterium pseudodiphtheriae or avirulent pneumococci) the reaction differed very little from that occurring when a nonliving irritant is injected intraperitoneally. Occasionally the neutrophil percentage was still high at twenty-four hours after injection, and it might remain relatively high (above 25 per cent) at forty-eight hours; in those cases in which the neutrophils remained high, the mononuclear cells rose less rapidly toward the normal percentage. It should be noted that cultures from the peritoneal fluid were positive at thirty minutes, one hour and usually six hours after injection but were never positive at twenty-four hours after injection (chart 1).

Effect of Virulent Bacteria.—When virulent pneumococci were injected intraperitoneally there was presented a different picture from that just discussed. It is advisable to separate the animals which succumbed to the infection into groups: (1) animals which died in a short period after injection, that is, within the first twenty-four to one hundred and twenty hours, and (2) animals which died after five days. This is principally justified because the reactions tended to be uniform within the earlier period but became more irregular in the later periods. It is not implied that there was an actual uniformity even in the periods through the ninety-sixth hour; the individual animals showed variations, but there was a tendency toward similarity of the reactions.

We may concern ourselves solely with the neutrophils and mononuclears since the eosinophils and lymphocytes were essentially negligible after the sixth hour subsequent to injection of the bacteria. By the sixth hour and occasionally at

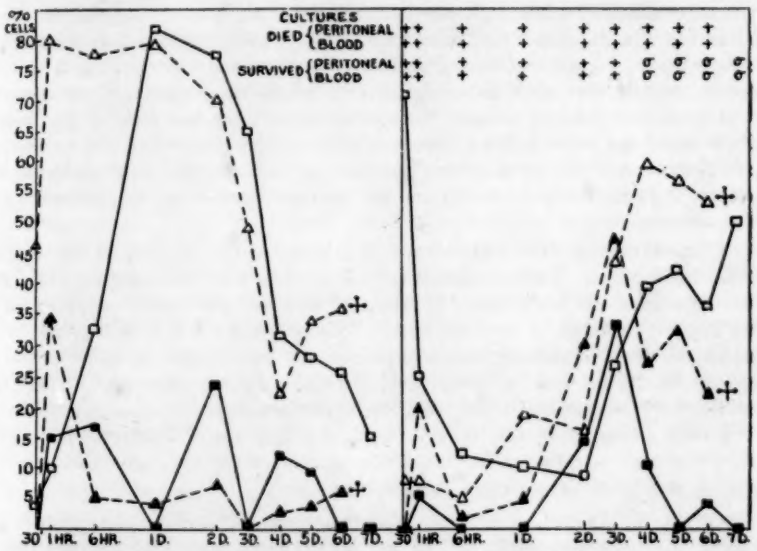

Chart 2.—Percentages of neutrophils, mononuclears and phagocytosing cells in peritoneal fluid of animals given intraperitoneal injections of virulent pneumococci. Solid line represents animals that survived; broken line, animals that died. Open figures show neutrophils (left side of chart) or mononuclears (right side); solid figures show phagocytosing cells.

one hour after injection the neutrophils had risen to 90 per cent or higher and continued at this level at the twenty-fourth hour and even on to the forty-eighth hour. Usually, however, by the forty-eighth hour there had occurred a drop in the relative percentage of neutrophils so that these cells might represent from 50 to 75 per cent of the cellular elements. Twenty-four hours later there might be a further fall which, however, rarely brought these cells below 40 per cent of the total, and at ninety-six hours the neutrophils might fall to from 20 to 35 per cent of the total cells. At times, however, there occurred a secondary rise of the neutrophils at the seventy-second or ninety-sixth hour, and even at later periods the neutrophils might remain about 75 per cent of the total cellular elements (chart 2).

The mononuclears fell rapidly and sharply in the first six hours (even at one hour these sometimes fell to from 7 to 25 per cent) and represented only about from 3 to 15 per cent of the cells. At twenty-four hours they might still be present in a low percentage, but often at this time there was evident a slight tendency for them to increase; the percentage at this time was, however, rarely above 25 per cent. After forty-eight hours the percentage might show a further rise to close to 50 as a maximum; however, at this period the mononuclears might still be low, representing only slightly over 10 per cent of the cells. At ninety-six hours their tendency toward an increase was more marked, the mononuclears usually varying between 30 and 65 per cent, and rarely was the percentage under the lower figure at this time.

If we consider those animals which survived for periods longer than one hundred and twenty hours, and in which the peritoneal cultures remained positive throughout the experiment, i. e., until the death of the animal, we find that the percentages of neutrophils and mononuclears were much as in the animals which died before five days, but that after this time considerable variation appeared in the percentages of neutrophils and mononuclears from day to day. It was noted in some animals that after an apparent reduction of the numbers of pneumococci in the peritoneal fluid on some of the earlier days (third and fourth) the neutrophils fell and the mononuclears rose in percentage, but that when the numbers of bacteria increased, the neutrophils rose and the mononuclears fell. Subsequently, however, the neutrophils decreased and the mononuclears increased up to the death of the animals.

Attention may be directed toward the lymphocytic reaction in these more extended infections. The percentage of lymphocytes rose with the early increase in mononuclears and later tended to remain high even while there occurred variations in the percentage of mononuclears. This tendency toward an increase in the lymphocytes was, in general, characteristic of the later stages of infection (at or after the fourth day whether the animal eventually died or survived). There was considerable irregularity in this reaction from one animal to another and even in the same animal from day to day. It is suggested that this increase in lymphocytic percentage is rather a factor of time than a factor associable with development of resistance or progress toward recovery.

Reactions in Animals Recovering Spontaneously.—A number of animals which received injections of pneumococci at the same time as those which served for the foregoing observations recovered spontaneously. In all animals considered in this group the peritoneal cultures were positive for the first few days after injection, usually through the fourth or fifth day after injection, but in some cases the positive peritoneal cultures persisted for longer periods. After the cultures were once negative they remained so throughout the period of observation (pneumococci might still be seen within the cells, however, for several additional days). The number of pneumococci in the peritoneal cultures was usually comparable to that in the cultures from animals which died, except possibly on the day before the peritoneal cultures became negative, when the number was at times reduced. In addition, in the cases in which animals recovered spontaneously blood cultures were positive in the earlier days and usually continued positive as long as did the peritoneal cultures (at times the blood cultures were negative twenty-four hours before the peritoneal cultures). It is apparent that in the animals which recovered there had existed a rather severe infection, and that recovery was not dependent on an initial power of resistance existing at the time of injection but must have depended largely on a resistance built up during the period of infection.

We can separate these animals into two groups: those in which the infection lasted only from four to five days and those in which the infection lasted longer. The majority belong to the former group; in the first three or four days both the neutrophilic curves and the mononuclear curves were essentially similar to those observed in the animals which eventually succumbed to the infection. At the fourth or fifth day, however, there was practically always a shift in favor of a high percentage of mononuclears and a low percentage of neutrophils in the surviving animals as compared with those which died. It must be remembered that at this period the bacteria either were about to disappear or had disappeared; and conditions were similar to those observed in animals receiving injections either of irritants or of nonpathogenic bacteria at from twenty-four to forty-eight hours after the injection. Except for the fact that the relations of neutrophils and mononuclears were restored toward normal with the disappearance of the bacteria, it was not possible to note any distinct difference in the cellular reactions in animals which died and those which recovered from the infection (chart 2).

Some animals which did not recover until after the fifth day showed positive cultures for from eight to ten days. In these animals there was a rather rapid rise in the percentage of mononuclears in the first few days of the infection (at forty-eight, seventy-two or ninety-six hours). At the same time one noted the relatively early fall in the percentage of neutrophils in the first few days. Beyond this period there occurred rather wide fluctuations in the percentages of neutrophils and mononuclears; this cannot be related to any demonstrable factor associated with the infection. It is possibly of interest to note that immediately before the disappearance of the bacteria (as demonstrated by cultural tests of the peritoneal exudate) the neutrophils seemed to show a definite increase in percentage which might either disappear as the peritoneal culture became negative or persist for a few days thereafter. Naturally, after the peritoneum no longer yielded positive cultures, the neutrophils fell (but only slowly), and in general the mononuclears rose toward their normal percentage. In these animals which eventually recovered one noted irregularities in the percental relations of mononuclears and neutrophils similar to those noted in animals which succumbed to the infection after a long period.

Reactions in Passively Immunized Animals.—Experiments were carried out in which guinea-pigs were passively immunized previous to the injection of the usual quantity of virulent pneumococci. Twelve hours before the injection of the bacteria antipneumococcic serum type I was administered subcutaneously (the intraperitoneal administration was not used so that irritation and resultant cellular reaction in the peritoneal cavity might be avoided). To one series of animals 3 cc. of antiserum was administered; three of four animals died. To a second series 3 cc. was administered subcutaneously twelve hours before injection, and an additional 3 cc. subcutaneously at the time of injection of the bacteria; all of the latter group survived. In the first series blood cultures and peritoneal cultures were positive during the life of the animals, except that in the case of the one which recovered the blood culture was positive at six hours but negative at twenty-four hours, and the peritoneal culture was positive at twenty-four hours but not thereafter. In the experiments in which larger amounts of antiserum were injected, both blood and peritoneal cultures were positive at six hours but negative at twenty-four hours (chart 3).

In spite of these differences in the course of infection the cellular reactions in the first forty-eight hours were essentially similar to those noted in other experiments in which bacteria were injected: The neutrophilic reaction was sustained, and the mononuclear percentage was depressed, tending however to rise. Again

there was noted the difference which appeared when the irritant (the bacteria) disappeared; that is, a sharp increase in the percentage of mononuclears and a fall in that of neutrophils.

PHAGOCYTIC ACTIVITY IN THE PRESENCE OF AVIRULENT PNEUMOCOCCI (ROUGH)

In four animals given injections of avirulent organisms the pneumococci were not demonstrable in the blood stream at any time, and they usually were absent from the peritoneal cultures at six hours after injection.

Thirty minutes after the injection, from 6 to 40 per cent of the neutrophils (present in relatively small numbers) had ingested bacteria. At one hour the

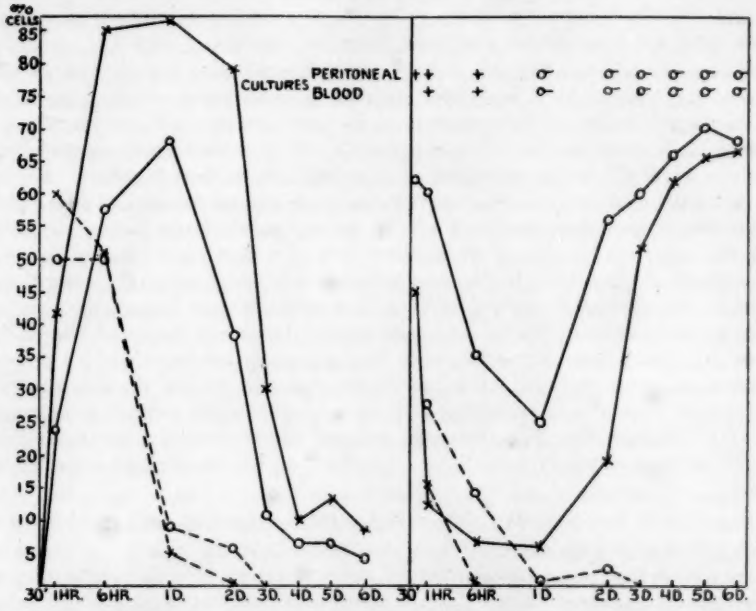

Chart 3.—Percentages of neutrophils, mononuclears and phagocytosing cells in peritoneal fluid of passively immunized animals given intraperitoneal injections of virulent pneumococci. Solid lines represent neutrophils (left side of chart) and mononuclears (right side); beaded lines, phagocytosing cells.

percentage of actively phagocytic neutrophils had fallen to between 0 and 6, and in most animals the phagocytic activity remained low or could not be observed; in two animals, one examined at four days and the other at five days after infection, bacteria was still seen in 10 and 20 per cent of the neutrophils, respectively.

In the earliest period (thirty minutes) the mononuclears showed relatively less phagocytic activity than the neutrophils; in one animal 10 per cent of the mononuclears had taken up bacteria while in the remaining ones from none to 3 per cent of the mononuclears showed phagocytosis. Usually at one and six hours there was no phagocytosis by mononuclears (one animal showed 5 per cent of the cells active at one hour). In most animals no phagocytosis by mononuclears was

evident at or after twenty-four hours, but in one animal 14 per cent were active at twenty-four hours, and in another animal very slight activity (from 2 to 1 per cent) was noted at three days and five days (chart 1).

It is apparent that phagocytic activity is most marked in the very early period after injection of avirulent pneumococci, but that this activity subsides rapidly simultaneously with the disappearance of the bacteria from the cultures.

While it has been stated that at thirty minutes the percentage of phagocytes is higher among the neutrophils than among the mononuclears, one should keep in mind the fact that at this time the number of mononuclears is definitely greater than that of the neutrophils, so it is probable that in the early stages the mononuclears are quantitatively actually the more effective in phagocytic activity.

Phagocytic Activity in the Presence of Virulent Pneumococci Reactions at from Thirty Minutes to Six or Eight Hours in Animals Which Died.—Within the period of the first six to eight hours the phagocytic activity of both neutrophils and mononuclears was quite irregular. In a considerable number of animals, probably about 50 per cent, no phagocytosis could be observed.

In a series of fourteen animals at a period thirty minutes after injection phagocytic activity by mononuclears (about 6 per cent) was observed in only one animal, and phagocytosis by neutrophils (about 13 per cent) in one other animal. At one hour, however, phagocytic activity could be noted in about from 20 to 25 per cent of the animals either by neutrophils or by mononuclears or by both. In the case of the neutrophils the percentage of phagocytes rose as high as 50 or was as low as 6; when phagocytosis was evident at this stage there was a tendency for the activity to be fairly marked. At this same period, in some animals, the mononuclears showed phagocytic activity, and usually it was less than that of the neutrophils.

At six hours there was a tendency for phagocytic activity to decrease, although in a few animals which had not shown any such activity of the neutrophils at one hour there was evident a marked degree of phagocytosis (in from 40 to 75 per cent); in other animals phagocytic activity was noted in 5 per cent or less of these cells. In the case of the mononuclears the tendency toward little phagocytic activity was more marked than in the neutrophils, and the majority of the animals showed no mononuclear phagocytes; three of the fourteen considered here showed 14, 4 and 4 per cent, respectively.

One must, of course, keep in mind (1) the fact that in these early periods the total number of leukocytes was usually small, except possibly at six hours, and (2) that this was a period of changing distribution of the types of cells—the mononuclears were falling and the neutrophils increasing in percentage. Thus if there was noted a high percentage of neutrophilic phagocytes at one hour, this may have represented little actual phagocytic effect; if there was neutrophilic activity at six hours, this may be considered more significant, but the significance would vary in individual cases. Since the mononuclears were decreasing in relation to the neutrophils, mononuclear activity was decreasingly less significant, and the low point of mononuclear phagocytic activity corresponded to the low point of the mononuclear percentage in relation to the total number of cells in the peritoneal fluid.

In comparing phagocytic activity in animals which eventually died and in animals which eventually survived it is not possible to note any difference at this stage; one may find relatively active phagocytosis or no phagocytosis in individual animals of each group.

Animals Dying Within Forty-Eight Hours.—In all of these animals phagocytic activity was marked at twenty-four bours, which was the last period of observation. This activity was evident in both neutrophils and mononuclears. In the case of

the neutrophils, which were, of course, the predominating cells, between 30 and 60 per cent had taken up bacteria. In the case of the mononuclears, which at this stage were relatively few, the percentage of phagocytes varied between 12 and 60. In both types of cells in most of the animals the tendency was toward the higher percentage activity.

Animals Dying Within Seventy-Two Hours.—In contrast with the group which died within forty-eight hours after injection, the animals which survived beyond the forty-eight hour period showed lower phagocytic activity at twenty-four hours. In the latter at twenty-four hours between 4 and 35 per cent of the neutrophils showed activity, usually less than 15 per cent; between none and 40 per cent of the mononuclears showed phagocytosis, with a rather large number of animals showing no phagocytes and the majority from no phagocytes to 10 per cent. However, at forty-eight hours these animals without exception showed a marked degree of phagocytic activity in both types of cells. Among the predominant neutrophils the percentage of the phagocytes varied between 65 and 95, and among the mononuclears, between 50 and 95 (with all but one animal showing a percentage above 65).

Both in animals dying before forty-eight hours and in those dying before seventy-two hours large numbers of pneumococcus pairs were found free in the peritoneal fluid, and in most cases the pneumococci practically carpeted the smear. It is evident, then, that the leukocytes had ingested only a small portion of the bacteria, but it was striking that in every case the phagocytic activity had been marked at a period shortly before death of the animals.

Animals Dying Within Six Days.—In the animals which survived longer than two days but which died within six days after injection of the bacteria the phagocytic activity within the first six to eight hours was very similar to that previously described and showed the same amount of irregularity. Nothing was observed in this earlier period in relation to the phagocytic reaction which differentiated these animals from those which died before the seventy-two hour period (chart 2).

At twenty-four hours the phagocytosis by neutrophils was usually negligible, often being unobservable, and rarely did more than 5 per cent of the neutrophils show this activity. At forty-eight hours, in the majority of the animals, phagocytic activity was low (still below 5 per cent), although in one animal 33 per cent of the neutrophils had taken up bacteria. From this time on until death the phagocytic action of the neutrophils was as a rule slight, the percentage of phagocytes being between 0 and 5 per cent, but an occasional animal would on one day, rarely on consecutive days, show a percentage as high as 33. On the day before death the neutrophilic phagocytosis was practically uniformly low.

The mononuclears tended to be more active than the neutrophils, but there was rather marked irregularity in different animals. Usually but not always phagocytic activity was evident at twenty-four hours, the percentage of the phagocytes rising as high as 45 in several animals, but in a few animals phagocytosis was absent from these cells or occurred in between 5 and 15 per cent. At forty-eight hours there was usually some phagocytic activity, the percentage of phagocytes in one animal rising as high as 30, but usually it was between 5 and 15; in a few animals at this period no phagocytic activity was observable. From this period onward mononuclear phagocytes were rarely absent, but in some animals the percentage seemed to vary from day to day between 5 and 40, tending possibly to center around 20 to 25. When the mononuclear phagocytosis had been high, a fall usually occurred on the day before death, but even at this time the phagocytic activity of the mononuclears was fairly marked. It should be remembered that in the latter days, third, fourth, etc., the mononuclears were generally tending to increase in

numbers even though with the continued presence of large numbers of leukocytes the neutrophils were probably the dominant cells.

Animals Dying After the Sixth Day.—In two animals which died after the ninth and before the tenth day certain rather similar tendencies were noted in the mononuclear activity but not in the neutrophilic activity. In one the percentage of neutrophilic phagocytes was increased markedly at the second and third days (from 55 to 60), later continuing at about 2 to 5, but falling to 0 at the eighth and ninth days. In the other animal the neutrophilic phagocytosis was negligible except at one day after injection. In both animals the percentage of mononuclear phagocytes was high (from 70 to 90) in the earlier periods, that is, through the third day. In the next few days it fell more or less gradually to a low of 0 on the seventh day in one animal and to 22 on the eighth day in the other animal. In both animals it rose to between 50 and 70 on the ninth day, which was the last observation before the death of the animals.

Animals Which Survived.—In animals which recovered spontaneously phagocytic activity in the early period after infection (from thirty minutes to six or eight hours) by either or both neutrophils and mononuclears was observed at times and was occasionally marked or was at times absent or almost negligible. The phagocytic activity of both neutrophils and mononuclears was essentially similar at the early period in animals which recovered and in those which eventually died.

At twenty-four hours the percentage of neutrophils that had ingested pneumococci was usually low, being 0 or rarely above 5, but that of mononuclears showing this activity was usually high (between 15 and 90), although such activity was absent in some animals. In general, the phagocytic activity in the group which survived corresponded to that which had been noted in guinea-pigs eventually dying after surviving beyond two days (chart 2).

At forty-eight hours there was often a tendency for the neutrophilic phagocytosis to be more marked than at twenty-four hours, but a number of the animals showed little phagocytosis at this period. Occasionally the percentage of neutrophilic phagocytes rose to 25, but usually it was below 5. The phagocytic activity of the mononuclears might now show a diminution when it had previously been high, the percentage of phagocytes falling to 5 or less; when it had been absent, phagocytic activity did at times rise, the percentage of phagocytes reaching a maximum between 25 and 40. Again in a few animals no phagocytosis by mononuclears was observed.

After forty-eight hours there occurred at times an increase of neutrophilic phagocytosis, which might be brief and variable or might extend over several days. In a number of animals some phagocytic activity of the neutrophils was noted at three days or over a period from the third through the sixth day, after which time, however, pneumococci were rarely seen in the neutrophils. In general, the mononuclears tended to show less phagocytic activity after forty-eight hours, and there occurred a more or less uniform tendency toward a decrease extending through the fifth, sixth and seventh day. In individual animals there were, however, irregularities in this descending curve, variations occurring from day to day. Usually in those cases in which the peritoneal culture was negative before or at the sixth day little or no phagocytic activity of either neutrophils or mononuclears could be noted at later periods; occasionally an isolated cell containing a few bacteria was found, but it might probably be considered as a cell which had failed to digest the bacteria taken up at an earlier period.

At later stages, from ten to fifteen days after injection, occasionally there appeared showers of mononuclears which contained numbers of bacteria, but these

appearances were usually brief in duration and were not accompanied by an increase in the number of neutrophils or any evidence of neutrophilic phagocytic activity.

In considering the phagocytic activity appearing in the animals which recovered spontaneously from the infection it was essential to bear in mind the fact that in these animals at a certain stage the peritoneal cultures became negative, in contrast to the fact that in the animals that died the cultures generally remained positive throughout. In a majority of the cases in which animals recovered, the cultures from the peritoneal fluid were negative after the sixth or seventh day; in a number of cases the cultures were negative after the third or fourth day. It should also be noted that during the first few days after the injection the blood cultures were also positive. It is apparent that with the disappearance of the pneumococci the phagocytic activity should fall. However, evidence of phagocytosis persisted for a few days even after the cultures of the peritoneal fluid were negative. This disappearance of bacteria from the peritoneal fluid must be given consideration when we compare the phagocytic activity in animals which survived with that occurring in animals which died.

Immune Animals.—In a group receiving only one injection of antiserum the blood cultures were positive at thirty minutes after infection, and in those which died both the peritoneal and the blood cultures were positive until death. In those animals which had received inadequate protection, the phagocytic activity at thirty minutes was negligible or absent as concerned both the neutrophils and the mononuclears, except in one animal (which did not survive). At one hour after injection of the bacteria there was in some cases slight phagocytic activity of the neutrophils, but this was absent in one half of the animals; the mononuclears in three of the four animals showed no phagocytosis. At six hours neutrophilic phagocytosis was more or less marked (between 5 and 25 per cent of the cells showing it), but mononuclear phagocytosis was absent in all animals. At twenty-four hours practically all animals revealed fairly active phagocytic activity (except one animal which showed no phagocytic activity of the mononuclears). From this time on until the death of the animals phagocytosis was more or less marked in all, and the usual high phagocytic activity was noted in the mononuclears at the last observation before death. In the case of the guinea-pig that survived the cultures of the peritoneal fluid were negative after twenty-four hours (the blood culture at twenty-four hours was negative), and the phagocytic activity of both types of cells fell sharply after twenty-four hours, although bacteria were seen in the cells (both neutrophils and mononuclears) even through the fifth day.

In the animals which were apparently adequately protected both the peritoneal and the blood cultures were positive at one hour but were negative after six hours and remained negative.

The phagocytic activity of both neutrophils and mononuclears was evident almost entirely within the first few hours. At one hour after infection between 50 and 60 per cent of the neutrophils (in one guinea-pig, however, less than 5 per cent) showed phagocytic activity, and between 5 and 30 per cent of the mononuclears showed this activity (the lowest percentage appearing in the same animal which showed only a small percentage of neutrophilic phagocytes). At six hours both the neutrophilic and the mononuclear percentage of phagocytes had fallen, the former to from 30 to 50 (in the inactive animal, to 0) and the mononuclear to 0 in three of the four animals and to about 4 in the fourth. At twenty-four hours no mononuclears showed activity, and the percentage of polymorphonuclears that did was between 8 and 0. After twenty-four hours the phagocytic activity had practically ceased, but a few cells, both mononuclear and polymorphonuclear, contained bacteria two days after injection (chart 3).

It is of interest to note the rather striking absence of phagocytosis in one animal of this series: Only at one hour after injection was slight phagocytosis noted (in about 5 per cent of both mononuclears and polymorphonuclears), and thereafter no phagocytic activity was noted. The cultures from this animal were identical with those from the remainder of the group.

COMMENT

It may be questioned whether this study of the cellular and phagocytic reactions during the course of infection by means of smears of the peritoneal fluid serves to give an adequate picture of the processes occurring in the body of the invaded host. Since the pneumococcic infection becomes a general infection shortly after the injection of the bacteria it is recognized that cellular and phagocytic processes must take place in other parts of the body. However, it may be assumed that this study gives a partial picture of the processes in the infected animal.

It is also to be noted as an essential limitation in these studies that the total number of cells in the peritoneal fluid during the course of the infection was not determined; it was impossible to determine this without killing the animals and thus defeating the possibility of determining the outcome of the infection.

It is also recognized that in the peritoneal cavity the omentum plays an important rôle in the course of infection.[2] A few animals were killed and smears from the omentum made at the early periods of infection with the idea of permitting a comparison between these smears and those of the peritoneal fluid. The changes in the distribution of cells at thirty minutes, one hour and six hours in the omental smears were essentially similar to those which had been noted in the free cells of the peritoneal fluid at the same periods. That is, the mononuclears decreased, the neutrophils increased, and the eosinophils and lymphocytes both diminished in numbers. As regards phagocytosis, at thirty minutes the phagocytic activity of the neutrophils and mononuclears was high, being observed in about 75 per cent of the mononuclears and 40 per cent of the neutrophils; at one hour the percentage of the mononuclears showing this activity had fallen markedly to about 55 and that of the neutrophils had risen to about 50. However, at six hours the percentage of neutrophilic phagocytes was between 15 and 20 per cent and that of mononuclear phagocytes was about 3. It seems apparent that the phagocytic activity of the wandering cells associated with the omentum is very much like that of the cells free in the peritoneal fluid. There is, then, justification for assuming that the study of the peri-

2. Durham, H. E.: J. Path. & Bact. **4**:338, 1897. Dudgeon, L. S., and Ross, A.: Tr. Path. Soc., London **57**:155, 1906. Buxton, B. H., and Torrey, J. C.: J. M. Research **15**:55 and 73, 1906.

toneal fluid gives a fair indication of what is occurring in the peritoneal cavity as a whole.

Through all these studies of the cellular response occurring in the peritoneal cavity subsequent to injection of irritant substances of various types one notes in the early stages the same sort of reaction. In large part, the response up through the first twenty-four hours is the same: An increase in the number of neutrophils begins to become evident at one hour after the injection and reaches a maximum sometimes at six hours and usually by twenty-four hours; a relative decrease appears in the mononuclears which is possibly actual in the first few hours; in addition, the lymphocytes tend to diminish in number or disappear; the eosinophils show a relative increase in the first few hours (probably not, however, an actual increase), but soon their number is negligible. This may be considered the routine response to introduction of an irritant. It is not assumed that in these first few hours the change in the cellular picture in the peritoneal cavity is principally an active one, but rather that the decrease in mononuclears is due to the adherence of these cells to the omentum.[2]

After this first twenty-four hour period the reaction depends on the persistence of the irritant. When chemical substances or nonpathogenic bacteria have been injected, the entire reaction tends to subside rapidly: The percentage of neutrophils falls, and that of mononuclears rises so that by forty-eight or seventy-two hours cells of the latter type dominate the picture; in addition, there may be noted in many cases a more or less marked increase of the lymphocytes. The eosinophils do not tend to return to the normal percentage for seven days or even longer, and neutrophils, which are either few or absent in normal peritoneal fluid, tend to persist for an equally long period.

If, however, the irritant, in the form of bacteria, persists in the peritoneal cavity, there is not evident the same tendency toward restoration of the normal relations of the cellular elements. The neutrophils tend to remain present in relatively large quantities for several days. Even though the infection persists, however, there may be noted the tendency for the relative number of neutrophils to decrease and for that of mononuclears to increase. There may occur irregular fluctuations in the relations of the two types of cells.

The tendency for the mononuclears to increase at or after the twenty-fourth hour is observed in all animals which survive for forty-eight hours. This increase occurs even though the infecting agent is present. It is possible that the increase in mononuclears is related principally to time and is not dependent on the stimulus (irritant), since in animals receiving injections of avirulent organisms the rise of mononuclears is also noted after a somewhat similar lapse of time. However, in the animals receiving injections of avirulent organisms the

increase in mononuclears is apparent earlier than in those receiving injections of virulent organisms; this difference may be apparent rather than real and may depend on the neutrophilic reaction resulting from the presence of virulent organisms masking the mononuclear increase. The assumption may be made that the neutrophilic reaction is essentially dependent on the irritant effect of the bacteria and their products, while in contrast the mononuclear reaction is largely dependent on time and in part independent of the irritant.

If one compares the cellular reactions in animals which succumbed to the infection with that in animals which recovered, there is practically no difference to be noted except, of course, in the recovering animals at the stage when the bacteria disappear from the peritoneal cavity. When the pneumococci have disappeared, the neutrophils tend to fall rapidly and the mononuclears to increase; it is certainly justifiable from the study of the cellular reactions in individual animals to assume that the direction which the reaction takes at this period is the result of the disappearance of the irritant rather than to assume that the pneumococci disappear as a result of the changes in the cellular elements.

If this interpretation of the cellular reaction is correct, there is nothing in the quantitative characters of the cellular reaction which can be related to either recovery or fatal termination. The cellular reaction in the peritoneal cavity cannot, then, be related to the processes of resistance to infection but must be assumed to be essentially determined by the factors of irritation or stimulation as exerted by the pneumococci.

If one compares the phagocytic activity noted in normal animals which succumb to the infection with the activity in normal animals which survive one finds very little difference between the two lots:

In the earlier periods through six or eight hours the same tendency toward fairly active phagocytosis may be noted in a considerable number of animals, irregular it is true, varying as to neutrophils or mononuclears, but definite in many animals. In a number of cases in this study both in animals which survived and in those which succumbed, no phagocytic activity by either type of cell was noted. It may probably be considered that in this early period before twenty-four hours after infection the phagocytic reactions in normal animals given injections of virulent bacteria are essentially nonspecific and not related to the possibility of death or recovery.

While usually both in animals which survived the infection and in those which died some time after the third day, phagocytic activity was depressed or absent at twenty-four hours or even occasionally at forty-eight hours, in those animals which died before the second or third day phagocytosis was usually marked at the last observation previous to the death of the guinea-pig. It may be suggested that the active phagocy-

tosis was due to the presence of large numbers of bacteria, but in view of the fact that enormous numbers were present on the slides of the peritoneal fluid of all animals at this stage it is not likely that this factor played a rôle.

At twenty-four hours the phagocytic activity of the neutrophils was, with the exceptions mentioned, usually at a low ebb both in animals which survived and in those which eventually died; at this time phagocytic activity of the mononuclears was usually evident, at times even marked, but occasionally absent.

After the twenty-four hour period the neutrophilic activity was usually absent, although an occasional guinea-pig showed phagocytic activity of this cell at the second, third or fourth day; this occurred in animals regardless of the later course of infection. In both groups of animals the mononuclear phagocytosis was quite marked on the second day and through the latter periods, usually tending to show a slight increase at the observation just preceding the death of the animal. The mononuclear phagocytosis in the later periods is all the more significant since the percentage of these cells in the peritoneal fluid was usually rising in the later periods.

When one compares the phagocytosis in animals which recover with that in animals which die, one must keep in mind the fact that in the former at a certain stage the bacteria are no longer demonstrable by culture in the peritoneal fluid. In discussing cellular reactions it has been stated that the effect of removal of the irritating agent should become evident in the cellular reaction, and in a similar fashion it is apparent that the disappearance of the bacteria should affect the phagocytic activity. If one compares the phagocytic activity in the animals which recover with that in the animals which die one finds up to the time of disappearance of the pneumococcus similar tendencies and similar variations. After the peritoneal cultures have become negative in animals which survive, the phagocytosis naturally falls.

If one considers the phagocytosis which occurs in the later stages of infection in practically all animals and especially that at the period shortly preceding death in animals which succumb to the infection it is difficult to determine whether the leukocytes are ingesting only bacteria which have lost their virulence or are ingesting bacteria regardless of virulence. Cultures of the bacteria obtained from the peritoneal fluid have not shown any rough colonies or any diminution of virulence for mice. Certainly, one would have to assume that at periods twenty-four hours or later after infection there should be present only virulent organisms, since by selection and adaptation the avirulent organisms should have been lost. The evidence presented suggests that in vivo, in the course of infection, the wandering cells of the host may and do ingest bacteria which are virulent.

The phagocytic activity of the eosinophils should be noted. In the early stages of the infection these cells are usually active; in many animals nearly 100 per cent of the eosinophils may contain bacteria. Usually these cells take up few bacteria; only one or two pairs of pneumococci are usually found within the eosinophil. This early phagocytic activity may be noted in animals which survive and in those which die, and is independent of the early neutrophilic or mononuclear phagocytosis. Like the general trend of phagocytosis the eosinophilic phagocytosis is not noted in some animals.

In the early stages of infection, before twenty-four hours, there is a tendency for both neutrophils and mononuclears to take up bacteria. With due allowance for the differences noted in individual lots and individual animals, it seems that at this stage, when the preexistent mononuclears are present and the neutrophils are beginning to pass out from the vascular system into the peritoneal fluid, both these types of cells possess phagocytic ability. After twenty-four hours there occurs a period in which nearly always the mononuclear cells show a considerable degree of phagocytosis, and the neutrophils only occasionally show it. In animals which die before forty-eight hours or seventy-two hours, however, the neutrophils show marked activity; in others surviving longer periods the neutrophilic action is only rarely noted. At periods beyond forty-eight hours the phagocytic activity in animals which die and in those which survive is chiefly confined to the mononuclears. This is, of course, not due to either absence or small numbers of neutrophils. It is evident that in the later stages of infection the neutrophils play but little rôle in phagocytic activity, and the mononuclears take over this function. It appears that although the phagocytic activity of neutrophils and mononuclears may be essentially equivalent when tested in vitro,[3] this is not the case in the course of infection; and at the later stages of infection the mononuclears are the active phagocytic cells even though neutrophils may be present in the peritoneal exudate in large numbers. In this connection one may recall the stress which Gay and his co-workers[4] laid on the clasmatocytes in resistance to streptococcic infection.

If our results represent a fair cross-section of the phagocytic activity in fatal infection and in infection with recovery, it cannot be stated that phagocytic activity as shown by the free cells of the peritoneal cavity is a factor in successful resistance developing during the course of infection.

If one compares the phagocytic activity in satisfactorily immunized animals with that appearing in normal ones (either dying or surviving),

3. Lucké, B.; Strumia, M.; Mudd, S.; McCutcheon, M., and Mudd, E. B. H.: J. Immunol. **24**:455, 1933.

4. Gay, F. P.; Clark, A. R., and Linton, R. W.: Arch. Path. **1**:857, 1926.

it is apparent that the reactions are rather different. In animals which are completely immunized practically all phagocytic activity occurs within the first six to twenty-four hours, and after this period few phagocytic cells are noted. To a certain extent this corresponds with the phagocytic activity noted when avirulent or nonpathogenic bacteria are injected into normal animals, but is more rapid and more complete. It is what one might expect as a result of sensitization of the bacteria through the presence of antibodies. It is possible that these differences in phagocytic activity between immunized and normal animals denote differences in the mechanisms leading to recovery, so that one cannot assume that the mechanism of resistance in the immune animal and that of resistance in the spontaneously recovered animal are identical. These differences may lie in the factors concerned in the two processes or may lie in the time and sequence of the various activities within the host, but the differences observed are too striking to pass over with simply the assumption that the activity of antibodies aiding in phagocytosis accounts for the apparent differences.

Since there is the suggestion that neither the cellular reaction nor the phagocytic activity can serve to explain the phenomenon of spontaneous recovery from infection, we have to seek for some other factor as the vital one concerned. Naturally, we turn to the concept of antibody activity, and this phase is now under investigation. It is interesting to note, however, that we did not observe in recovering animals the increase of phagocytic activity with the approach of recovery which may be expected if the tropic activity of antibodies is the vital one concerned. Furthermore, in a few animals which were apparently highly resistant to pneumococcic infection (animals which had been rendered immune with antiserum or which had recovered from infection and later were again infected with pneumococci) the peritoneal fluid, after injection of the bacteria, remained sterile even at thirty minutes, and no phagocytosis and very little cellular reaction were noted in this fluid at any time. Thus in the highly immunized animal evident reactions, at least as concerns the free cells in the peritoneal cavity, were absent. Either the mechanism of resistance is associated with immediate engulfment of the bacteria and fixation of the bacteria on the walls of the peritoneal cavity (the omentum), or cellular reactions are negligible factors in the resistance. It may be noted that the reactions related to clearing up of the infection in these animals were more striking and proceeded more rapidly than did the reactions in normal animals given intraperitoneal injections of nonpathogenic bacteria, again suggesting that the mechanisms operating in natural immunity, in active immunity and in spontaneous recovery may be differentiated either in toto or in the temporal sequence of the factors concerned.

In support of the concept that phagocytic activity may be of secondary importance is the observation of Mackie, van Rooyen and Finkelstein [5] that, in the presence of serum, leukocytes possess a bactericidal activity separable from their phagocytic activity.

It is of interest, finally, to note that in the animals which recovered spontaneously the time of recovery as measured by the disappearance of pneumococci from the cultures of the peritoneal fluid varied between the third and seventh day after infection. Excluding one case in which the cultures were negative at the second day and one in which the cultures were not negative until the tenth day, we find four cases in which the cultures became negative on the third, three on the fourth, four on the fifth and one on the sixth day after infection. It seems that this tendency for the crisis (if it may be so called) to appear before the seventh day of infection is of some significance and must be given consideration in further studies.

SUMMARY

In studying the cellular reactions in the peritoneal cavity of the guinea-pig subsequent to intraperitoneal infection with Pneumococcus type I it was not possible to note any differences between animals which died and animals which recovered spontaneously or any differences in the cellular reactions which served to explain the mechanism of recovery.

It has not been possible to observe in the wandering cells of the peritoneal fluid any difference in phagocytic activity of neutrophils or of mononuclears as between animals that survived spontaneously and those that died. This applies to the amount of phagocytosis and to the sequence of the activity.

The question is therefore raised as to whether phagocytic activity plays a rôle in spontaneously arising resistance to infection.

The results suggest that the process of resistance in immunized animals and that in animals spontaneously recovering from infection are not identical. This may be due to a difference in the factors concerned or simply to differences in the intervals of time and the sequences.

It does not appear that the phagocytic activity of neutrophils and that of mononuclears are identical in the various stages of infection. The neutrophils appear to lose phagocytic activity in later stages while the mononuclears retain their activity.

5. Mackie, T. F.; van Rooyen, C. E., and Finkelstein, M. H.: J. Path. & Bact. **39**:89, 1934.

Case Reports

DWARFISM ASSOCIATED WITH LINGUAL GOITER AND CYSTIC HYPOPHYSIS

H. Gideon Wells, M.D., Chicago

As far as I have been able to learn from a search of the literature on dwarfism, the following case is unique in some respects, and hence it is placed on record.

REPORT OF A CASE

A woman, aged 72, was examined post mortem at the Oak Forest Infirmary on Oct. 25, 1933, four days after her death in this institution, the poor-farm of Chicago and Cook County, where she had lived for twenty-three years. It was impossible to find any members of her family or any one who knew anything about her early life. Her name indicated that she was probably of Irish descent. According to the records she was admitted to the Oak Forest Infirmary in 1910 because of feeblemindedness; she came from the Chicago State Hospital, where she had been since 1896, at which time her age was given as 35. She was quiet and able to act as doorkeeper in the ward for mentally defective women. No history was obtainable. A roentgen examination could not be made because of the patient's obstinate attitude. It was reported that she was in a failing condition because of weakness without special symptoms in April 1933, but she was not bedfast until August 1933. She died on Oct. 21, 1933; there were no clinical records of significance during her period of stay at the Oak Forest Infirmary.

No records were available as to her previous history. She never spoke unless spoken to, and her replies were difficult to understand (possibly because of the lingual goiter). She was decidedly substandard mentally; she did not read or write, and although she was able to dress and undress herself and to take care of her physical needs she did not change to clean clothes unless she was told to. Nevertheless she was able to act as doorkeeper in the irresponsible women's ward, and she guarded the door jealously, never permitting patients to escape but admitting the proper attendants. She could not be made to retire until the physician had made the night rounds. When crossed she showed a violent temper and scolded vigorously but unintelligibly. No persons had visited her in many years, and nothing could be learned about her family and relatives. There were no records at the Chicago State Hospital. Dr. Ralph Hamill wrote as follows concerning her behavior while in that institution: "I remember a dwarf that I think is the one you refer to, although I should be surprised if that woman had lived as long as this. The patient I refer to was in the poorhouse. She was used as a messenger by some of the nurses and could be counted on to deliver messages. However, she chuckled much to herself, passed remarks on the side that were not a part of the message and obviously lived some kind of an imaginary life quite independent of the reality. There are no records that I know of, and I know nothing of her family or early history."

From the Department of Pathology, University of Chicago, and the Otho S. A. Sprague Memorial Institute.

It is to be regretted that no study of her basal metabolism was ever made, but as she lived as an abandoned mentally defective person on a county poor-farm she was not made the subject of a clinical study, and she outlived her association with those who knew her early history.

Postmortem Examination.—The body was that of an extremely emaciated white female dwarf, somewhat short-limbed, appearing to be at least 80 years of age. Practically she was nothing but skin and bones. The typical face of the achondroplasic dwarf was lacking (fig. 1), as the nose was retroussé but fairly sharp and had a drawn appearance because of the prominence of the inner end of each malar bone. The distance between the eyes was somewhat increased because the glabella was disproportionately broad. The skull was approximately symmetrical, but the

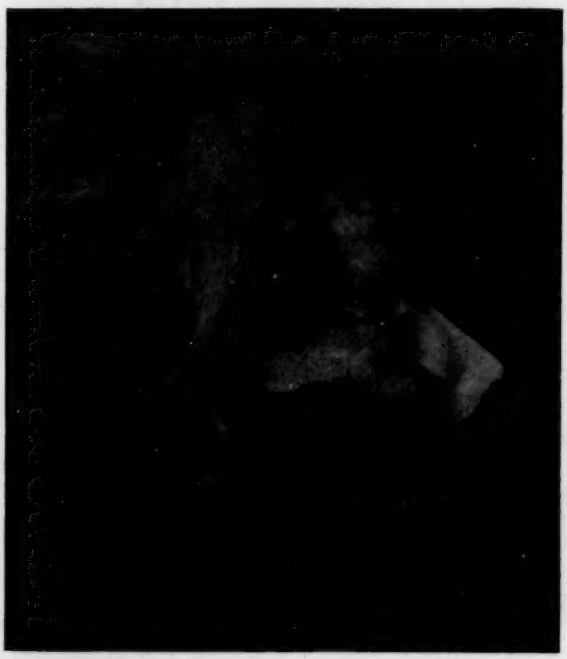

Fig. 1.—Profile of dwarf showing absence of cretin characteristics.

left eyebrow was 0.5 cm. higher than the right. The skull was not rachitic. The posterior portion of the skull cap was somewhat flattened, and the skull was of less than normal size.

The external measurements of the body were taken. The total length, standing, was 124.5 cm. (49 inches), and sitting, 72 cm. (28⅜ inches); the total weight eviscerated, 29¼ pounds (13.33 Kg.); the calculated weight (total), from 39 to 40 pounds (17.7 to 18.1 Kg.); the length of the body (from the upper edge of the manubrium to the lower edge of the symphysis), 46 cm.; the total length of the upper extremity, 50.5 cm., and the transverse width of the shoulders, 26 cm. The lengths of various bones were as follows: humerus (from the top of the head to the lower border of the external epicondyle), 21.8 cm.; radius (from the tip of the styloid process to the top of the head), 16 cm.; ulna (from the tip of the

styloid to the tip of the olecranon process), 18.4 cm.; carpus, 2.7 cm.; third metacarpal, 4.3 cm.; third proximal phalanx, 4.5 cm.; third middle phalanx, 2.8 cm.; third distal phalanx, 1.8 cm. (a close approximation). The total length of the lower extremity (from the anterior superior spine to the styloid) was 57.2 cm.; that of the femur (from the upper extremity of the greater trochanter to the outer margin of the surface of the joint), 29 cm.; the length of the tibia from the surface of the joint to the styloid process measured 23.5 cm.; the total length of the fibula was 24 cm. The patella measured 4.7 by 4.2 cm. The total length of the foot was 18.5 cm. The measurements of the pelvis were as follows: intercristous, 25.5 cm.; interspinous, 23.5 cm.; baudelocque, 18 cm., and bitrochanteric, 27.5 cm. The measurements of the head were as follows: biparietal, 13.5 cm.; bitemporal, 11.6 cm.; fronto-occipital, 16.6 cm.; occipitomental, 23.5 cm.; maximum anteroposterior, 20 cm.; maximum vertical, 20 cm.; circumference, 53 cm.

The hands were small in proportion to the feet and were not of the trident type. The fingers were slender and graceful, not clubbed. The teeth were missing, and the alveolar processes were absorbed. The superficial lymph nodes and the thyroid were not palpable. The mammary glands were very small but apparently had been slightly developed; the areolae were devoid of pigmentation. The skin was extremely atrophic (apparently more so than is usual in senile persons); there was no growth of hair on the axillae or on the body, with the exception of a few hairs in the pubic region. The joints of the upper extremities all moved freely, but the hips were restricted in adduction by fibrous bands which could be stretched readily. The knee joints were in a fixed semiflexed position but could be extended under pressure. The pelvis was relatively roomy, the distance between the anterior superior iliac spines being 24 cm. The external genitalia were normally formed but were hypoplastic. There were protruding hemorrhoids. There were decubitus ulcers over the sacrum, both great trochanters and the left fifth metatarsal bone.

The large serous cavities showed no significant changes. The immediate cause of death was apparently asphyxia, as the larynx, trachea and main bronchi were all completely plugged by a solid mass of granular material, apparently regurgitated cereal. The smaller bronchioles were filled with pus, and there was bronchopneumonia in the posterior half of the right lung.

Internal Organs: Most of the viscera showed no changes except those associated with the dwarfism and with senility. The heart weighed 180 Gm.; in the lower part of the interventricular septum it showed extensive fibrosis due to atherosclerosis of the coronary arteries. The aorta showed moderate senile atherosclerosis. The liver weighed 560 Gm. and showed merely small hepatic cells and slight fatty changes. The spleen weighed only 30 Gm.; it contained an abundance of lymphoid tissue; the reduction in size was apparently due to loss of the pulp. The pancreas weighed 60 Gm. and showed no changes. The cortex of the suprarenal glands was poor in lipoids; the medulla was normal. Both suprarenal glands were small and of normal shape, and the right gland measured 30 by 16 by 4 mm.; the left, 37 by 13 by 4 mm.; each weighed approximately 2 Gm. As seen microscopically the cortex was unusually poor in lipoid, but the cellular structure and arrangement seemed entirely normal. The medullary cells contained an abundance of brown pigment. The capsular arteries were not excessively sclerotic. A few rather large accumulations of round cells were present in the medulla. The kidneys together weighed 140 Gm. and appeared to be of nearly normal appearance except for occasional small cysts, and although the medium-sized arteries were much thickened the arterioles were not affected, and there was very little evidence of arteriosclerotic scarring. The cortex seemed almost normal. The

ureters and urinary bladder were normal. No abnormalities were found in the alimentary canal. The lymph nodes in general were normal for the age, one peribronchial node being calcified.

Reproductive Organs: The generative organs seemed to have reached an early stage of complete maturity but had apparently retrogressed or ceased to develop shortly after early puberty, for there were a few pubic hairs, and a microscopic examination of the ovaries showed occasional corpora albicantia, indicating that there had been some ovluation, but most of the ovary was devoid of such scars and consisted of homogeneous ovarian stroma. There was one small follicular cyst. Grossly the uterus was of distinctly infantile character, the corpus measured 3 cm. and the cervix also was 3 cm. long and formed more of the entire uterine mass than the corpus, the entire uterus being 6 by 4 cm. The opening of the cervix was of normal size, and its canal was equal in length and diameter to that of the uterus. The cervical canal showed virginal markings. The whole organ appeared small. The ovaries were extremely small and of equal size. They measured 1.5 by 0.7 by 0.5 cm. There was no gross evidence of corpora albicantia. The vagina was normal except for a few small round pigmented spots. The hymen was intact.

The whole of the external genitalia seemed to share in the extreme emaciation of the body, and the perineum and perineal structures were tightly stretched over the bony pelvis. The adipose tissue of the mons had almost totally disappeared, as had the adipose tissue of the labia majora. The skin of the region shared in the general atrophy of the skin and subcutaneous tissues that was so marked everywhere else over the body. There were a very few fine brown hairs over the mons veneris and a few more along the outer side of the labia majora extending backward as far as the anus. The labia majora were atrophic and covered with shiny, thin, atrophic skin, although the mucocutaneous junction was recognizable. They were well developed anteriorly. There was no posterior fourchet, and the labia were lost in the perineum while widely separated. The perineum was 4 cm. long, flattened and covered with thin, shiny, atrophic skin. The labia minora were small but well developed, 15 cm. long and 0.9 cm. wide. They united in a normal manner anteriorly to form the frenum of the clitoris and blended with the labia majora posteriorly opposite the middle of the vaginal opening. The glans clitoris was very small and was barely recognizable since it was covered with a large, well developed, unusually thick but apparently normally formed prepuce. The vaginal opening, gaping and distorted by postmortem examination, measured 2.2 cm. in its greatest diameter.

Macroscopic Examination of the Structures of the Neck: Of particular interest was the condition of the thyroid, which seemed to exist only in the form of a lingual goiter. The tongue had a flat, atrophic mucosa. Immediately below and to the left of the foramen caecum was a globular submucous swelling, approximately spherical and 2.7 cm. in diameter. When incised this mass contained an encapsulated brownish nodule of soft tissue about 1.5 cm. in diameter, not presenting the appearance of thyroid tissue and showing no visible colloid or cysts. The thyroid gland was absent from the normal position. When the lingual mass was opened from beneath it was found to be disk-shaped, measuring 2.5 by 2.3 cm. by 1 cm. It could be freed from its dense fibrous capsule with relative ease by blunt dissection, excepting over its dome, where it was attached to the lingual mucosa by tissue as hard as cartilage. Its cut surfaces showed an irregular mixture of deep yellow-brown glandular tissue with irregular masses of firm glistening grayish-white tissue. There was no recognizable colloid. Over the dome of the mass the pale fibrous type of tissue was preponderant. Elsewhere the fibrous

areas were scattered irregularly throughout the center of the mass, and the periphery contained brown glandular tissue. There was no similar-appearing tissue either along the midline anterior to the thyroid cartilage or at the usual site of the lateral lobes.

The dissection of the organs of the neck by Dr. Eleanor Humphreys disclosed that on either side of the trachea were two peculiar small bodies (fig. 2) well encapsulated and easily demarcated from the adjacent tissue. The larger, on the right, was an L-shaped body. The pointed upper end of the long arm of the L reached almost to the base of the first tracheal cartilage and was 1.5 cm. long. The blunt, rounded short arm, pointing laterally, was 1 cm. long. Below the pointed upper end the width of the arm varied from 3 to 5 mm., and the thickness averaged 3 mm. The upper end was composed of solid pale tissue. Below this was a part in which a translucent cyst, about 3 by 2 by 2 mm., occupied one side of the long arm, crowding some brownish firm tissue to the side adjoining the trachea. The rest of the mass was a flabby, brownish, finely lobulated structure, resembling atrophic fat.

The mass on the other side was similar in general features, but it was smaller and more V-shaped. Both arms were relatively short and thick, and the tip of the lower was occupied by a cyst. When this was cut, a small solid, free body like a rice grain escaped. This was about 2 by 2 by 3 mm. Each arm measured about 1 cm. in length by 3 by 3 mm. This mass lay at a slightly lower level than that on the other side.

A search of the tissues on both sides of the trachea did not reveal any discrete pale bodies resembling the parathyroid glands.

Microscopic Examination of the Tissues of the Neck: The microscopic examination of the tissues of the neck showed no substance resembling thyroid tissue; the presence of such tissue was limited to the lingual mass. This mass consisted mostly of necrotic fibrous tissue in which nuclei were scanty and evidences of previous glandular structure difficult to detect. Blood pigment was seen in many areas. Only at the periphery were traces of recognizable thyroid tissue to be found, this consisting of a thin zone of atrophic acini, from one to six acini deep, sometimes empty and sometimes containing a small accumulation of colloid. The epithelial cells lining these acini were scanty; they were entirely absent in many of the acini, and when present they were generally very small, exhibiting as a rule little more than a small pyknotic nucleus and no recognizable cytoplasm. There were also acini that were indicated chiefly by an outline with little recognizable structure. The general appearance resembled that often seen in old fibrosed adenomatous nodules in which frequent hemorrhage and necrosis have occurred until the adenoma has been nearly completely destroyed. However, there was no marked calcification or ossification such as is often seen in such fibrosed adenomas.

Two normal, well developed parathyroid glands were present, one on each side of the larynx (R 2 and L 1, fig. 2). In the vicinity of each parathyroid gland were small cysts lined with a few layers of flattened epithelium which was not keratinized. No more complex epithelial structures were found, nor anything resembling thymus tissue; possibly the epithelial structures represented undeveloped embryonic anlage of the thymus. There was also some adipose tissue, which was of the adult type and not like "brown fat."

The carotid gland was found on the left side by means of serial sections made through the vicinity of the bifurcation of the carotid. It seemed to be small and diffuse but otherwise normal. The right gland was not sought.

The pineal gland appeared to be normal for the patient's age; it contained numerous calcific bodies.

No thymus tissue could be detected in the usual site.

ADDITIONAL EXAMINATION

Structures of the Neck.—Dr. George M. Curtis, who examined the tissues removed from the neck, thought that the nodule found medial to the bifurcation of the right carotid showed a resemblance to thymus tissue. The lingual thyroid, he stated, "is characteristic of the rudimentary thyroid gland found in the typical dwarf cretin."

As to the other structures in the neck designated in figure 2, Dr. Curtis reported the following observations:

Right L-Shaped Body (fig. 2): Section R 1 (proximal end) revealed alveoli and several cysts with a lining of stratified squamous epithelium. The picture was compatible with the early embryonic thyroid gland. On the other hand, since the thyroid was mainly lingual in its origin and had not descended, I should regard this tissue as having originated from one of the brachial epithelial bodies.

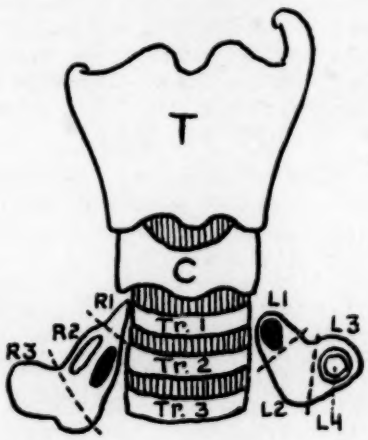

Fig. 2.—Diagram of neck structures in case of lingual goiter.

Section R 2 (midportion containing cysts) was a parathyroid gland. I interpreted it as such because of the characteristic arrangement of the cells in cords interspaced by blood-filled sinusoids.

Section R 3 (distal end) showed no glandular elements. Areolar and adipose tissue and vessels were evident.

Left L-Shaped Body (fig. 2): Section L 1 (proximal tip) was a characteristic parathyroid gland. The cordlike arrangement of the cells, the intervening sinusoids and the characteristic general arrangement convinced me that this was one of the parathyroid glands.

Section L 2 (midportion) showed no glandular elements and was composed of areolar and adipose tissues; it also contained vessels.

Section L 3 (distal tip containing cyst) again showed alveoli which were compatible with embryonic thyroid alveoli. Again, however, I regarded this tissue as having originated from the branchial epithelial bodies. From which ones, it is impossible to ascertain.

Section L 4 ("rice body" from the cyst in L 3) was colloid and had no particular cytologic characteristic.

I believe that the thymus, thyroid and parathyroid glands are definitely accounted for in these sections. The unusual alveoli and glandular elements in sections R 1 and L 3 may have been derived from the so-called "lateral thyroid anlagen." This derivation is by no means clear. It would be safe, however, to call them derivatives of the branchial epithelial bodies. I regard the cysts as having originated from branchial epithelial body anlagen.

The absence of other thyroid tissue in cases of lingual thyroid is probably common in view of the frequency of myxedema following removal of the lingual growth. According to Ungermann,[1] six of eight patients examined at autopsy had no thyroid tissue other than that found in the lingual growth.

Dr. Curtis had an analysis made of the lingual thyroid tissue in his laboratory by Francis J. Phillips and reported as follows:

The gland as removed from the preservative fluid weighed 3.6 Gm. It was desiccated in a vacuum desiccator at 40 C. The weight after desiccation was 0.476 Gm. Three determinations made on this desiccated gland showed an iodine content of 31.6 mg. per hundred grams of desiccated gland. The preservative fluid from the gland was likewise analyzed in triplicate. The average result of these analyses was 0.168 mg. per hundred cubic centimeters of fluid. Therefore the entire gland contained 0.25 mg. of iodine.

This amount is to be contrasted with that found in the normal thyroid, which is about 2 mg. of iodine per gram of dry weight, or from 10 to 15 mg. in the entire gland.[2] As a control a portion of skeletal muscle from the dwarf was also analyzed, and no detectable amounts of iodine were found. It is of interest that in the thyroid from a typical achondroplasic dwarf I was unable to find any iodine by a method capable of detecting 0.05 mg. of iodine,[3] whereas in the thyroid from a giant examined by Bassoe [4] I found 62.9 mg. of iodine.

No important changes were found in the brain, which was examined by Dr. Percival Bailey. The hypophysis, however, was greatly altered, consisting of a soft, thin-walled cyst, which collapsed when the brain was removed. The sella turcica was slightly enlarged but otherwise normal. There was no evidence of change in the floor of the sella.

When examined microscopically the hypophysis was represented only by the collapsed wall of the cyst as obtained at the autopsy and consisted in part of the capsule surrounding the gland. The structure was not a definite cyst with a wall lining the cavity but rather a fibrous wall consisting of the capsule of the hypophysis and the adjacent membranes, within which lay fragments representing different elements of the hypophysis. There were many small masses representing the anterior lobe, in which the cells seemed to be flattened into rows by pressure. There was an abundance of posterior lobe tissue, and the structures of the pars intermedia were recognizable in close relation to the pars nervosa. The cellular elements of each of these segments showed the histologic characteristics of a normal

1. Ungermann: Virchows Arch. f. path. Anat. **187**:58, 1907.

2. Wells, H. G.: Chemical Pathology, ed. 5, Philadelphia, W. B. Saunders Company, 1925, p. 690.

3. Hektoen, L.: Am. J. M. Sc. **125**:751, 1903.

4. Bassoe, P.: Tr. Chicago Path. Soc. **5**:231, 1903.

pituitary as usually seen in bodies dead for several days. The impression was that the cyst had been produced in the anterior lobe by simple softening. There was nothing indicating any sort of neoplastic process or of hypophyseal duct cysts. There was no evidence of inflammation, either acute or chronic.

The skeleton was examined with roentgen rays by Dr. Paul C. Hodges, who reported the following results:

Roentgenograms of the pelvis and the lower part of the abdomen were taken with the subject in the supine position on grid visualization from the upper lumbar

Fig. 3.—Skeleton of dwarf with lingual goiter, showing absence of usual cretin characters, slight relative shortening of limbs, and well developed female pelvis.

part of the column halfway to the knees. I suspected a congenital abnormity of the upper sacral and lower lumbar segments. I looked for unfused epiphyses but found none. The bones were small. The pelvis was somewhat deformed. The femoral heads were slightly mushroomed, and the necks were short. A solitary bony island was present in the neck of the left femur. Some of the vessels were calcified. Roentgenograms of the upper part of the trunk were taken with the subject in the supine position on grid visualization of both shoulder joints, the elbow joints, most of the ribs and the vertebrae from the sixth cervical to the fourth lumbar. No lesions were seen. There were no unfused epiphyses. The

teeth were all missing. The bones, of course, were very small for a subject of the patient's age. The roentgenogram of the skull was taken with the left side against the film. The region of the pituitary gland showed the effect of the removal of bone at autopsy. The bones of the skull were rather thick. The teeth had all been removed. The roentgenogram of the knee was taken with the lateral surface against the film. There was marked wasting of the soft tissue. Calcification of the arteries was present above and below the knee. The articular ends of the femur and tibia had a rectangular blocklike appearance, and the articular cartilage appeared to be thin. No osseous lesion and no unfused epiphyses were observed. The roentgenogram of the left ankle was taken with the lateral surface against the film. There was generalized osteoporosis which may have been due to disuse or to senility. The peripheral vessels were calcified. There were no unfused epiphyses. In the left hand, wrist, forearm and elbow no lesions were observed except for wasting, calcification of the blood vessels and smallness. In the right hand and forearm, of which roentgenograms were taken with the palm down, there was evidence of an old Colles fracture of the distal end of the radius and of the ulnar styloid. Except for this, the appearance was the same as in the corresponding parts on the left.

After the skeleton had been separated from the soft tissues and mounted it was found to be characterized by the small diameter and delicate appearance of all the bones (fig. 3). Relative shortness was evident in the extremities as in the entire body. The old healed fracture of the right radius was the only recognizable deformity. There was no evidence that the epiphyseal lines had failed to close, and there was no suggestion of rachitic deformity. The alveolar processes of the jaws had been entirely absorbed.

COMMENT

As dwarfism may result from either thyroid or hypophyseal deficiency, it is difficult to determine the relative rôle played by each condition in this case, as a history of the early life of the patient and metabolic or other clinical studies are lacking. As Erdheim [5] has pointed out, both the thyroid gland and the hypophysis are necessary for normal endochondral ossification, for the presence of thyroid tissue alone when the hypophysis is destroyed or the presence of the hypophysis alone with the thyroid destroyed does not suffice to produce normal endochondral ossification. Erdheim believed the influence of the hypophysis to be greater than that of the thyroid gland, since in hypophyseal dwarfs the lines of ossification remain open in more bones and up to a later age than in cretins; however, cretins do not usually live very long, while a total loss of the hypophysis may permit of a longer life. Essentially, the hypophysis and the thyroid gland have the same effect on the three phases of endochondral ossification. Peritz stated that the growth tendency at birth seems to be adequate to carry the development of stature only to from 130 to 140 cm.; beyond this height the influence of the hypophysis is required.

Athyreosis alone, despite a normal-appearing hypophysis, may lead to marked dwarfism. Janney [6] says that some of the most severe cases of dwarfism known have occurred among cretins. These dwarfs usually

5. Erdheim: Beitr. z. path. Anat. u. z. allg. Path. **62**:303, 1916.

6. Janney, in Barker, Lewellys F.: Endocrinology and Metabolism, New York, D. Appleton & Company, 1922, vol. 1, p. 391.

show a low degree or almost complete lack of intelligence, as contrasted with the good intelligence usually exhibited by the proportionate dwarfs, and cretins usually show a marked underdevelopment of the genital system.

According to Paltauf [7] the skeleton of the cretin is characterized by short, thick tubular bones which exhibit a coarseness in surface structure. The epiphyseal ends tend to be thick, low and flat, so that the bone scarcely possesses a true neck. The head of the femur is flattened and the neck short, producing the usual waddling gait. The base of the skull is typically short, as Virchow described it, with complete synostosis between the occipital and sphenoid bones, and the anterior and posterior sphenoid centers are fused. These fusions produce the typical broad, flat nose with depressed root usually exhibited by cretins as well as by achondroplasic dwarfs.

Although the patient under discussion undoubtedly had defective thyroid function and presumably was a cretin, the anatomic observations were not typical of cretin dwarfs as described by Paltauf, for the skeleton was well formed and graceful; the base of the skull was not shortened, and the facies generally observed in cretins was not present except for an increased width of the glabella, which measured 31 mm. from orbit to orbit. At the advanced age of this woman the lines of ossification had completely closed and seemed normal. While in hypophyseal dwarfs the epiphyseal lines remain open for a long time, they do eventually close, as in the remarkable case reported by Priesl,[8] that of a dwarf measuring 132 cm. who lived to be 91 years old; at autopsy the epiphyseal lines were found closed; the anterior hypophyseal lobe was a thin-walled cyst as in my case, but the thyroid was present in the usual site, weighed 15.9 Gm. and showed a normal histologic picture according to the age of the dwarf.

As far as is known there had been no myxedema in the case here reported despite the scanty amount of thyroid tissue present. While the extremely atrophic condition of the skin at the time of death suggested the previous existence of some trophic deficiency in the skin, premature senile changes of the skin (geroderma) have been described in hypophyseal dwarfs.

The skeleton in this case showed no severe disproportion. Although the arms and lower extremities were short in proportion to the trunk, this was not extremely marked, and did not resemble the disproportion observed in achondroplasic dwarfs. In this respect the conformation resembled more that of the hypophyseal dwarfs, who are classed with the proportionate dwarfs, than that of the cretin dwarfs who are classed with the achondroplasic and rachitic among the disproportionate dwarfs. However, the limbs were definitely short in proportion to the trunk, for, as can be seen from the photograph, the finger-tips reached but little below the great trochanters. The lower extremities bore about the same relation to the trunk as did the upper extremities. There were no traces of rachitic changes in the bones.

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7. Paltauf, Arnold: Ueber den Zwergwuchs in anatomischer und gerichtsarztlicher Beziehung, nebst Bemerkungen über verwandte Wachsthumsstörungen des menschlichen Skelettes, Vienna, Hölder, 1891.

8. Priesl, A.: Beitr. z. path. Anat. u. z. allg. Path. **67**:220, 1919; Wien. med. Wchnschr. **80**:589, 1930.

There seem to have been few observations of dwarfism in association with lingual goiter, which is of itself an infrequent condition, and none of the cases previously reported had been studied post mortem. In 1914 Asch [9] was able to collect but ninety-five cases of lingual goiter recorded in the literature to that date; of these only 12 per cent were in males. In his discussion of the literature Asch made no mention of dwarfism as associated with lingual goiter, although a few such cases have been reported. Walther [10] reported the operative removal of a lingual goiter from a *zwerghalft kleine Frau* of imbecile appearance, who had lost the power of speech at 12 years because of the lingual tumor and had been confined in an institution thereafter. But he gave no measurements or other details and did not state the results of the operation. This case apparently was in some respects like the one here reported.

Another similar case has been reported by de Boncourt.[11] This report concerned a dwarfed female cretin 122 cm. tall, aged 37, with the apparent mental development of a child of 10, who had much difficulty in speaking and some trouble in breathing. The goiter was removed, but further events were not reported. Other cases of retarded growth associated with lingual goiter, but also without autopsy observations, have been reported by Urban,[12] Harvey,[13] Krassnig [14] and Rebattu.[15] All these dwarfs with lingual goiters were females.

In 1921 Kohl [16] collected ninety-three cases of lingual goiter—of which eighty-three occurred in females—often giving brief abstracts of the cases in addition to the titles, but no further mention of dwarfism could be found among these reports.

Whether the dwarfism in the case under consideration was due entirely to the thyroid deficiency or partially or entirely to the hypophyseal abnormality cannot be determined. Against the assumption that that lesion was responsible is the character of the hypophyseal lesion, which seemed to be merely a central softening of unknown etiology. There remained an amount of anterior lobe tissue which according to its microscopic appearance might well have been adequate for the needs of the small body. Nor did the microscopic appearance suggest that this lesion was of sufficient age to correspond with the period of fifty-five years since the developmental stage of the subject. Simmonds [17] has called attention to the fact that to cause dwarfism destructive lesions of the anterior lobe must occur during the years of growth; later in life they cause cachexia. In most of the few established cases of hypophyseal dwarfism the responsible lesion has been a cystic tumor of the hypophyseal duct as

9. Asch, R.: Deutsche Ztschr. f. Chir. **130**:593, 1914.
10. Walther: Beitr. z. klin. Chir. **77**:116, 1912.
11. von Chamisso de Boncourt, A.: Beitr. z. klin. Chir. **19**:281, 1897.
12. Urban, Karl: Zentralbl. f. Chir. **50**:701, 1923.
13. Harvey, F.: Brit. J. Surg. **13**:746, 1926.
14. Krassnig, Max: Ztschr. f. Laryng., Rhin., Otol. **12**:113, 1924.
15. Rebattu: Presse méd. **31**:904, 1923.
16. Kohl: Schweiz. med. Wchnschr. **51**:361, 1921.
17. Simmonds: Deutsche med. Wchnschr. **45**:487, 1919.

described by Erdheim. However, in Priesl's 91 year old dwarf the anterior lobe of the hypophysis was described as consisting of only a thin membrane with scanty parenchyma rests, similar to the hypophysis in my case but differing in the presence of an osseous defect in the sphenoid with a persistent craniopharyngeal duct. Simmonds made the assumption, without any supporting evidence, that in this case the anterior lobe had been destroyed by embolism; but the presence of the persistent craniopharyngeal duct suggests the probability that this had something to do with the condition.

As Sternberg [18] has pointed out, infantile dwarfs are not necessarily hypophyseal, for the proportionate dwarfs with hypothyroidism must be considered as an independent group of nanosomia infantalis. There is also a third group of dwarfs in whom both the hypophysis and the thyroid are normal or at least not responsible for the defects of growth, and these persons are characterized by hypoplasia of the sex glands, whereas in dwarfs with hypophyseal and thyroid deficiency the sex glands atrophy because of endocrine deficiency. Obviously the hypoplasia of the sex glands is not responsible for dwarfism, since eunuchs are tall. There seems to be no constant relation between the state of the hypophysis and that of the thyroid in cretinism according to the reports in the literature (Falta). When the thyroid is removed there is a tendency for hyperplasia of the anterior lobe, but a report of a cystic condition such as that seen in my case could not be found as a sequel of thyroidectomy.

Perhaps the case most like the one under consideration in respect to the hypophysis is that reported by Kraus and Holzer [19] of a female cretin dwarf, 40 years old, 115 cm. tall, in whose hypophysis was found a large irregular cyst involving one third of the anterior lobe, in which the parenchyma had been replaced by a thin colloid-like fluid containing many basophil cells. This cretin had a thyroid in the normal site showing much fibrosis and adenomatous areas. Death followed a cesarean section with delivery of a normal child. All the epiphyseal lines were closed, and the skeleton was well proportioned except for the relative shortness of the lower extremities. However, in this case the large skull and sunken nose of cretinism were present. Kraus and Holzer considered that the hypophyseal cyst formation was probably not responsible for the dwarfism; they believed that it was relatively recent and secondary to the thyroid deficiency, and they called attention to the fact that Bayon and von Stefanoff observed an increase in the hypophyseal colloid in cases of thyroid aplasia. Although their case is more definitely one of typical cretinism than the case reported here the two cases together indicate that cystic changes in the hypophysis may result from a high grade of thyroid deficiency.

All things taken together, it seems probable that this case is primarily one of dwarfism due to hypothyroidism, in which the functional thyroid tissue is reduced to a minimum amount in a greatly degenerated lingual goiter, there having been no formation of thyroid tissue at the normal sites. As is usual in these cases, the parathyroids are normal and the suprarenal glands, while very small, do not show definite abnormalities.

18. Sternberg, C.: Beitr. z. path. Anat. u. z. allg. Path. **67**:275, 1919.

19. Kraus and Holzer: Virchows Arch. f. path. Anat. **251**:253, 1924.

Apparently sexual development ceased at about puberty, a few ovulations perhaps having taken place. However, it is to be noted that the skeletal changes were not those of typical cretinism, and in some respects the condition resembles "pituitary infantilism" as described by Berblinger,[20] characterized by a defective development of the anterior lobe of the hypophysis and hypoplasia of the genital organs, with normal development of the other endocrine glands. In these cases adiposity is absent because of the lack of involvement of the brain.

SUMMARY

The case here reported is that of a mentally substandard female dwarf, 72 years old, 49 inches (124.5 cm.) tall, weighing about 40 pounds (18.1 Kg.), who had been in institutions for the care of the incompetent and impoverished for thirty-seven years and on whom no clinical study had been made. No history of her youth or family could be obtained. Death was due to an impaction of food in the larynx, trachea and bronchi, perhaps because of the obstruction to regurgitation caused by a lingual goiter, measuring 2.5 by 2.3 by 1 cm. No other thyroid tissue could be found in the neck or elsewhere. This lingual thyroid consisted mostly of necrotic fibrous tissue, apparently the result of organization of old hemorrhages and involuted thyroid tissue. Only at the periphery of the nodule were traces of recognizable thyroid tissue found, and this was very atrophic. In it was found but 0.25 mg. of iodine, as compared with from 10 to 15 mg., the amount present in a normal thyroid. The chief other observation was a cystic degeneration of the anterior lobe of the hypophysis, of unexplained etiology and undetermined significance. Presumably this case is one of dwarfism due to hypothyroidism associated with incomplete cretinism; it is improbable that the hypophyseal cyst was responsible for the dwarfism, although the hypophyseal influence cannot be excluded.

20. Berblinger, W.: Beitr. z. path. Anat. u. z. allg. Path. **87**:233, 1931.

Laboratory Methods and Technical Notes

A SIMPLE APPARATUS FOR FIXATION OF LUNGS IN THE INFLATED STATE

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Ordinarily, the introduction of fixing solution (formaldehyde or Kaiserling I [1]) into the bronchi inflates the lungs satisfactorily, but unless the inflated condition is maintained evenly only the dependent portions (containing the greater part of the fixing solution) are permanently inflated, the remaining parts becoming fixed in partial inflation of uneven character. A method employing negative pressure for maintaining complete uniform inflation within physiologic limits has been worked out in this laboratory and has proved practicable for most purposes. The principle of this method depends on the maintenance of a certain degree of inflation of the lung for a number of hours, during which time the fixative may act.

The lung to be inflated must be removed from other structures, viz., the heart, pericardium and diaphragm, with the least possible injury to its surface, either by instruments or by the tearing of adhesions, in order to avoid leaks. As much of the bronchus as possible should be left on the lung, though a length of 1 cm. may suffice. One end of a short metal tube is inserted into the bronchus as far as it will pass easily, as shown in the figure. The tube should be about ½ inch (1.27 cm.) in diameter and from 2 to 4 inches (5.08 to 10.16 cm.) long; its other end should be threaded (*J*) for connection with a metal joint (*F*). To attach the lung to this tube it has been found necessary to adopt the following procedure: The stump of bronchus is grasped at one part of its edge by a narrow clamp, which is held in the same hand as the metal tube previously inserted; this prevents the tube from slipping out of the bronchus. A chain of elastic rubber bands looped end to end to make a total length of from 18 to 24 inches (45.7 to 60.9 cm.) is used to tie the bronchus on the metal tube (*K*). As this is wound about the bronchus constant tension is maintained; the clamp should not be included in the winding and may be released after a number of turns. By means of the constant elastic tension a surprisingly satisfactory joint is made between the bronchus and metal tube, and, in our experience, this has never slipped or leaked.

An ordinary large bell jar (*G*) is employed for the negative pressure chamber. Since it is made of glass, the stages of inflation of the lung may be studied, and roentgenograms may be taken at any stage. The jar is supported in a wooden base (*I*) with a concavity made on the lathe to fit the convexity and knob of the bell jar exactly. The cover of the chamber is made of a broad, thick disk of wood (*D*) with a circular groove (*E*) cut near its edge to fit easily over the rim of the jar; the groove should be wide enough to permit the cover to be removed

From the Laboratories of the Mount Sinai Hospital.

1. This solution consists of 30 Gm. of potassium acetate, 10 Gm. of potassium nitrate, 750 cc. of distilled water and 300 cc. of a solution of formaldehyde.

easily. An air-tight seal is made by partly filling the groove with petrolatum just before it is applied to the jar. An opening is cut directly in the center of the cover for a large-sized rubber stopper (*C*), through which, in turn, a threaded metal tube (*B*) is inserted snugly and made to project far enough below the under surface of the cover so that the metal joint (*F*) may be screwed on. The lung is attached to the joint by means of the other metal tube (*J*) previously described, after the bronchus has been tied on. Another opening is cut in the cover near one edge to admit a small glass Y-tube (*A*), one arm of which is connected with a suction pump, the other with a simple water manometer (*H*) by means of a flexible rubber tube. The Y-tube with its connections is inserted after the cover with the suspended lung is in place, and is removed when the cover is to be taken off.

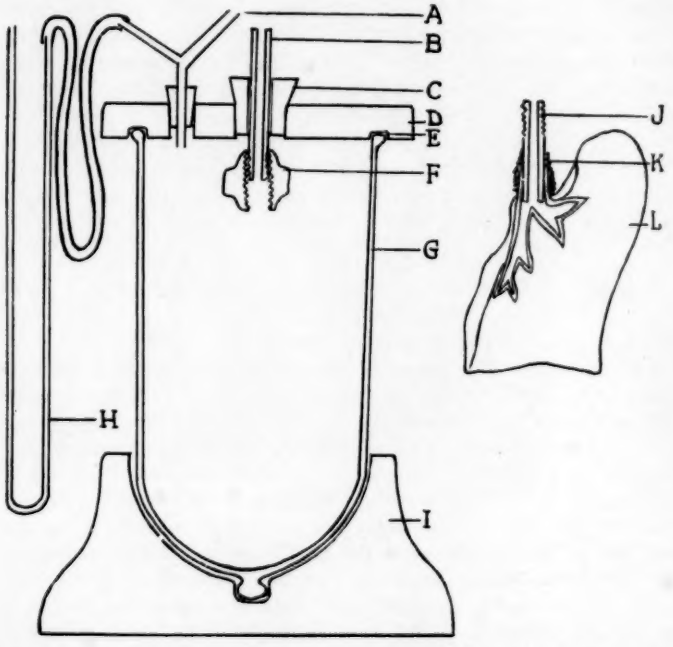

Diagram of bell jar and connections employed in maintaining inflation of lung to be fixed. *G* indicates the bell jar, which is supported in the wooden base *I* and is covered with a broad thick disk of wood *D*, with a circular groove *E*. *C* is a rubber stopper, through which a threaded metal tube *B* is inserted and made to project far enough below the surface so that the metal joint *F* may be screwed in. The lung is attached to the latter by means of the tube *J*, one end of which is inserted into the bronchus, which is tied on the metal tube *K*. *A* is a small glass Y-tube, one arm of which is connected with a suction pump, the other with a simple water manometer *H* by means of a flexible rubber tube.

With the lung in place, suction is applied (from 10 to 20 cm. of water) until the inflation reaches its maximum throughout; the entire lung should expand uniformly, unless pathologic alterations are present or the bronchus is blocked by improper fitting of the metal tube. Illuminating gas may now be run into the

bronchus through *B* to fix the hemoglobin of the lung a permanent red. Fixing solution (Kaiserling I) is then introduced into the bronchus by means of a funnel joined to *B*; this is added slowly until a total of from 2 to 3 liters has been used. Ordinarily, a considerable amount is lost through surface tears and through the blood vessels of the hilus, and a slight amount through surface transudation from the pleura. While the fixing fluid is being added all leaks are rendered temporarily air-tight, so that a marked rise in negative pressure usually takes place, requiring adjustment of the suction pump repeatedly. When the addition of fixing fluid is completed, it is necessary to regulate the suction until a stable reading for negative pressure is reached. Suction should be left on from twelve to eighteen hours.

In the presence of rents in the pleura, it may be possible to produce a serviceable seal by searing the torn surface with a hot iron until it is perfectly dry and applying liquid adhesive reenforced with cotton wool; this must be permitted to dry completely.

When a lung fixed in this manner is opened it appears somewhat dry on cut section; its color is delicate salmon pink; the normal alveoli are distinct, and the bronchi and bronchioles appear as smoothly rounded tubes which retain their shape. If a portion of lung tissue is squeezed, even to the thinness of paper, it still retains its capacity to reexpand, and if placed in water swells and regains its former appearance immediately.

This method is not suited to diseased lungs in which it is desired to maintain atelectasis. On the other hand, it is ideal for such conditions as bronchiectasis, bullous emphysema, suppurative pneumonia and tuberculosis.

Microscopically, the alveoli appear unusually well inflated and almost emphysematous, yet with intact walls, even when inflated with extremes of pressure. The finer architecture of the lung is much more distinct than is usually seen, even in lungs removed under ideal circumstances from healthy persons dying suddenly. It is possible to distinguish the complete ramification of the bronchioles and alveolar ducts in almost diagrammatic perfection. The alveolar septums appear thinner than usual and somewhat elongated; their capillaries show corresponding changes. Edema fluid has a foamy appearance on section, as though churned violently with the air used for the inflation. Pneumonic exudate appears looser than usual, and the inflamed alveolar septums are more clearly defined, often showing leukocytes in passage from capillaries into alveolar interstitial spaces and alveolar lumens. The inflamed alveolar septums often appear slightly edematous, revealing a distinct interstitial space containing fibrin and blood cells. The interstitium is not apparent in uninflamed alveoli, even when greatly congested. The lymphatics, in general, are rendered more prominent; with prolonged inflation and the use of large amounts of fixing fluid they may be emptied of exudate or tumor cells. (This observation may possibly possess some significance in relation to the physiology of the circulation of lymph within the lung.)

By means of overdistention with fixing fluid and prolonged inflation under high negative pressure, a unique microscopic picture is produced. The alveolar architecture is preserved; the alveolar septums, however, are extremely thin and stretched practically straight in places, so that

many septums lie flat instead of being perpendicular to the surface of the slide, appearing as narrow ribbons. Wider ribbons are seen, with sections of greater thickness (e. g., 20 microns). It is thus possible to study the surface of the alveolus in strips. In our experience with this method we have never found pores of Cohn [2] to exist in normal alveoli. Elastic fibers are numerous and alveolar epithelial cells are seen singly in places lying on the surface. There is no evidence of non-nucleated squames (von Kölliker).

2. Pores of Cohn, or Cohn's stomas, are minute apertures in the walls of the pulmonary alveoli. Whether they actually exist preformed, permitting the passage of air, edema fluid or fibrinous exudate from one alveolus into another, or whether they are artefacts has not been determined.

General Review

EXPERIMENTAL CHOLESTEROL ARTERIOSCLEROSIS AND ITS RELATIONSHIP TO HUMAN ARTERIOSCLEROSIS

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I. INTRODUCTION

In the experimental study of arteriosclerosis in animals the most painstaking efforts have been directed toward the production of arterial lesions capable of really close comparison with those of human arteriosclerosis. The lesions produced in the arteries of rabbits by cholesterol feeding represent the nearest approach to success in this direction. It is well known that arterial lesions can be produced quite easily in rabbits by a variety of other methods, but with these other methods the alterations in the walls of the vessels are confined almost entirely to the media. The intima is only secondarily affected, and the changes in it are relatively insignificant. Slight intimal thickenings of a purely fibrous character may be found but not with any regularity. These experimental lesions of the arteries can hardly be said to resemble those of ordinary human arteriosclerosis, in which thickening of the intimal layer is perhaps the most constant and conspicuous feature. Moreover, in human arteriosclerosis, the intimal thickenings are seldom composed of fibrous tissue alone; they are almost constantly the sites of more or less abundant accumulations of various lipoid substances. Intimal lesions of this nature, so familiar in human arteries, have been very closely imitated in the cholesterol feeding experiments which I propose to review in this paper. A complete and exact experimental reproduction of human arteriosclerosis in animals has not yet proved possible.

In rabbits, the feeding of large quantities of cholesterol or of foods rich in cholesterol results in the development of widespread lesions of the arteries. These lesions are characterized by a thickening of the intima and by abundant accumulations of lipoids[a] in the intima and the inner layers of the media. I have chosen to call this experimental disease of the arteries "experimental cholesterol arteriosclerosis." Cholesterol feeding as a method of producing fatty lesions in the arteries of rabbits had its origin in some experiments reported in 1908 by Ignatowski, who found the typical arterial lesions in rabbits which had been fed for some time with eggs and milk. The general resemblance between these lesions and the lesions typical of human arteriosclerosis was immediately recognized and aroused great interest. The obvious opportunities for investigation offered by the new experimental method gave rise to numerous studies of experimental cholesterol arteriosclero-

(*a*) Following Peters and Van Slyke,[119] I shall apply the term "lipoid" to all those substances in the organism which, in their general chemical and physical properties and especially in their solubilities, resemble the true fats. The latter are included also. The words "fat" and "fatty," unless otherwise specified, will be used in a loose sense to indicate especially those lipoids which can be stained with ordinary "fat stains," such as sudan III.

sis. It is chiefly to the results of these studies and to a consideration of their significance in relation to human arteriosclerosis that this paper will be devoted.

It should be mentioned at once that the effects of cholesterol feeding are not confined to the arteries; the lipoids accumulate also in various other tissues and organs throughout the body. It is not the intention here to describe the extra-arterial changes in detail or to enter into a discussion of their significance except so far as they are concerned in the interpretation of the lesions in the arteries. The possible applications of the experimental data to problems other than that of arteriosclerosis have been discussed fully by Versé,[165] Kawamura[70] and Chalatow.[32] These applications are really rather limited. Certainly it is safe to say that the most important inferences which have been drawn from the cholesterol feeding experiments are those relating to the etiology and pathogenesis of arteriosclerosis in man.

Even in the early lesions of human arteriosclerosis, the presence of lipoids in the intima can be easily recognized by routine histologic methods, and for this reason, the rôle of fatty substances in the development of arteriosclerosis has always occupied a prominent place in discussions of the pathogenesis of the disease. However, there are other alterations in the arterial walls which are not so readily demonstrated under the microscope and which may be even more important than the accumulation of lipoids. It has never been satisfactorily established from the study of human material that the lipoids play the major rôle. Whether they represent cause or effect is still a matter of controversy. On this question, more than on any other, the experimental results have been brought to bear as presumptive evidence of the great importance of the lipoids in the etiology and pathogenesis of human arteriosclerosis.

My interest in the cholesterol feeding experiments was aroused by the claims of many authors that experimental cholesterol arteriosclerosis in rabbits is virtually identical with human arteriosclerosis. In my own experiments certain features of the pathologic picture seemed to be rather inconsistent with such a conclusion. However, a more complete and thorough examination of the literature showed that my results differed in no essential respect from those of previous investigators. The discrepancy lay in the fact that the points of difference between human arteriosclerosis and the experimental disease have been almost always completely subordinated to the features of similarity.

The experimental data which have now accumulated have come to assume considerable importance. On the assumption of identity between experimental and human arteriosclerosis, the experimental results are frequently cited as evidence in support of a modification of the so-called "imbibition" or "infiltration" theory of human arteriosclerosis. This

theory, which was first proposed many years ago,[b] is offered in a somewhat different form as an explanation of the development of experimental cholesterol arteriosclerosis. It is assumed that the modified theory may be applied quite as appropriately in the explanation of the development of arteriosclerosis in man. The modification consists chiefly in the great emphasis which is laid on hypercholesteremia as a factor of primary etiologic significance in human arteriosclerosis. Such unreserved acceptance of the experimental data as evidence capable of direct application in the human being has led to inferences of far-reaching importance, which have attained widespread credence and popularity through the extensive writings of numerous authors. It seemed worth while, therefore, to examine rather carefully the experimental work on which these ideas are based, with the purpose of determining what facts have been established and of evaluating their importance as applied to the solution of problems of arteriosclerosis in man.

II. ORIGIN AND DEVELOPMENT OF CHOLESTEROL FEEDING EXPERIMENTS

In 1908 Ignatowski,[c] studying the effects of high protein diets on rabbits, noticed the occurrence of fatty plaques on the intimal surface of the aorta in some of his animals, as well as fatty accumulations in the liver and kidneys and marked enlargement of the suprarenal glands. The feedings had consisted of meat, eggs and milk, and he attributed the pathologic changes to the effect on a herbivorous animal of a diet rich in animal proteins. In the following year, Starokadomsky[d] studied the lesions in the arteries more carefully and described them fully. He had given his rabbits a diet of eggs and milk, and he, too, believed the lesions to be due to the toxic effect of the proteins in the diet. However, the experiments of Stuckey,[152] reported in 1910, threw some doubt on this point. He fed rabbits with milk, egg-white, egg yolks and meat juice in various combinations and found that the diets containing egg yolks produced marked fatty changes in the aorta and some of its large branches, while milk, egg-white and meat juice alone had no damaging effect on the arteries. Stuckey[153] was later successful in producing identical lesions by feeding brain tissue. These findings, together with other considerations, led to the idea that the lesions in the arteries and elsewhere were not due to the protein fraction of the diet but rather to some lipoid constituent common to egg yolks and brain tissue.

(*b*) References 15, 16 and 124 in bibliography.

(*c*) 64, 65, 66.

(*d*) 147, 148.

The experiments of Wesselkin [e] with the feeding of lecithin, although only 1 animal was given a really large amount, strongly indicated that this substance was not responsible for the pathologic alterations. Stuckey [153] had already shown that diets containing neutral fats and fatty acids were ineffective in producing the arterial lesions. It remained for Anitschkow and Chalatow,[f] in 1913, to demonstrate that the feeding of pure cholesterol [g] dissolved in oil could produce in rabbits lesions identical with those described by previous investigators. Independently and almost simultaneously, Wacker and Hueck [h] reported that pure cholesterol in solid form added to the ordinary food of rabbits could produce the lesions, and they showed further that it brought about a marked increase in the cholesterol content of the blood, an increase which was dependent for the most part on an elevation of the cholesterol esters.

Since that time similar experiments with various modifications have been carried out on rabbits by numerous investigators who have studied and described fatty accumulations in a variety of situations throughout the body. This work has demonstrated that the lipoids, which are for the most part anisotropic, are deposited first in the cells of the reticulo-endothelial system in the liver, spleen, lymph nodes and bone marrow. The cells of the suprarenal cortex and the central cells of the liver lobules become heavily loaded with lipoids. Later, fatty deposits occur in the arteries, in the valves of the heart and in the veins; in the skin, subcutaneous tissue and tendons; in the mucosa and submucosa of the bile ducts, gallbladder and stomach; in the interstitial tissue of the kidneys, and in the eyes, simulating the arcus senilis.

The effects of cholesterol feeding have been studied also in a variety of animals other than the rabbit, but attempts to produce arterial lesions in other animals by this method have met with limited success. Bailey [19] in 1915 reported the production of experimental cholesterol arteriosclerosis in guinea-pigs, and his results have since been confirmed by several other investigators. It is now clear enough that cholesterol-rich diets are capable of producing arterial lesions in guinea-pigs as well as in rabbits, but there has been no convincing demonstration of a similar effect in any other animal.

The literature bearing on the changes produced in various organs and tissues by cholesterol feeding has been well summarized by Schön-

(*e*) 178, 179.

(*f*) 4, 13.

(*g*) This and subsequent references to "pure cholesterol" indicate preparations of "cholesterol" supplied by commercial firms or manufactured by the experimenter. It is not to be inferred that such preparations consisted of a single pure chemical. In all probablity they contained two or more sterols of which one was almost certainly ergosterol.[119]

(*h*) 166, 167.

heimer.[135] More comprehensive publications in this field are those of Kawamura [70] and Chalatow.[32] The literature dealing especially with experimental cholesterol arteriosclerosis has been summarized briefly by Schultz,[138] and quite recently Anitschkow [12] has written a full review of the subject from the Russian point of view.

III. METHODS

A variety of cholesterol-rich diets have been administered in various ways by different investigators for the production of experimental cholesterol arteriosclerosis. Egg yolks have been the most commonly used of cholesterol-rich foods,[i] but brain tissue,[j] liver [k] and hydrous wool fat [l] have been used with success. These substances were mixed with the ordinary food (in the case of liver, as a dry powder), or were thinned out by mixture with some liquid and administered by means of a stomach tube. After the discovery that cholesterol alone would produce the same results, it was commonly employed. Solid cholesterol has been given mixed with oats or bread [m] or administered in capsules.[36] Others have used an oily solution of cholesterol, with which meal or bread was saturated.[n] Still others have introduced the cholesterol in oily solution directly into the stomach by means of a stomach tube,[o] and this seems to be the method of choice. A variety of mediums for the solution or suspension of cholesterol have been tried with success. Sunflower-seed oil,[p] cottonseed oil,[q] olive oil [r] and 5 per cent sodium oleate [100] all seemed to be suitable, and none of them alone produced changes in the arteries. The cholesterol content of the preparations ranged from 2 to 7.5 per cent.

The daily administration of the cholesterol ration through a stomach tube seems to be the most convenient method. The only valid objection to the use of a tube is the occasional loss of an animal from perforation of the esophagus or stomach or from aspiration pneumonia. However, both of these accidents can be avoided by the use of a soft tube, by practice in passing the tube and by care in cleaning it thoroughly before it is passed. It has been pointed out that the insertion of the stomach tube might cause an increase in blood pressure. Thölldte [154]

(*i*) 6, 7, 20, 42, 45, 67, 76, 152, 189, etc.
(*j*) 153, 156, 157.
(*k*) 38, 75, 76, 132, 156.
(*l*) 34, 109, 141, 142, 143, 169.
(*m*) 74, 131, 166, 167.
(*n*) 20, 42.
(*o*) 4, 5, 13, 45, 99, 100, 145, 189, etc.
(*p*) 4, 5, 13.
(*q*) 19, 20.
(*r*) 45, 100, 167.

has advanced evidence to the contrary, but with this possibility in view, some investigators have preferred to avoid the use of the tube and have given cholesterol or cholesterol-rich substances mixed with other foods. The objections to this method are the uncertainty as to how much cholesterol the animals actually ingest and the occasional difficulty encountered in inducing them to take the food.

Whatever method is chosen for the introduction of cholesterol, its administration must be continued for some time, and its total quantity must be considerable before the lesions in the arteries make their appearance. If solid cholesterol is fed, its administration must be continued for a longer time and its total quantity must be greater in order to produce the arterial lesions than is the case if it is employed in solution in oil or as it occurs naturally in egg yolks, hydrous wool fat, etc. Thus, Anitschkow [s] found the earliest microscopic changes in the aortas of his rabbits after from 30 to 45 days when a total of from 11 to 18 Gm. of cholesterol in oil had been given by mouth. The earliest gross alterations were seen at the end of from 55 to 78 days after a total of about 30 Gm. of cholesterol had been fed. Bailey [20] observed early changes in the aortas of his rabbits only after the administration by the oral route of 14.5 Gm. of cholesterol in oil in 36 days, or of 26 egg yolks in 30 days. On the other hand, Wacker and Hueck,[t] who fed their rabbits with solid cholesterol were unable to find any changes in the arteries in less than 5 months, although the daily dose was 1.25 Gm., the total quantity of cholesterol therefore being in the neighborhood of 175 Gm. or more. Apparently the more rapid development of lesions after the feeding of cholesterol in solution is dependent on the greater ease with which it is absorbed in this form from the gastro-intestinal tract.

Some investigators have combined the administration of cholesterol-rich diets with other procedures designed to injure the arterial walls simultaneously. These procedures have included the use of the suspension method of Klotz,[72] narrowing of the aorta by a loose ligature, injections of epinephrine hydrochloride or of certain toxic substances, or even direct injury to the wall of the aorta by cauterization. Others have modified the experiment by interference with the endocrine system with the object of altering the cholesterol metabolism in such a way as to favor or retard the accumulation of lipoids in the organs and arteries. These special methods will be mentioned later with their results.

At this point it may be remarked that the concentration of attention on the cholesterol content of the diet seems to have detracted from any serious consideration of its general nature. The diet, apart from the

(*s*) 4, 5.
(*t*) 166, 167.

added cholesterol, has usually consisted of foods commonly used for the maintenance of laboratory animals and, of course, has been the same as that given to control animals. Nevertheless, it seems much more desirable that the diet, except for its excessive content of cholesterol, should be a balanced one which is known to be adequate in all respects. In this connection, the work of Blumer and his associates [26] is suggestive, purporting to show that chlorophyll added to the diet retards the development of experimental cholesterol arteriosclerosis in rabbits. The effects of added substances other than cholesterol (e. g., the other lipoids and the proteins in egg yolks, the oils used for the solution of cholesterol, etc.) must also be taken into account.

A number of investigators have experimented with parenteral administration of cholesterol. By the use of intravenous injections of a preparation of cholesterol in 5 per cent sodium oleate, Klotz [73] was able to produce arterial lesions in rabbits which he described as corresponding to the early stages of the lesions occurring in the cholesterol feeding experiments. Dewey [40] reported similar results following intravenous or intraperitoneal administration of a colloidal suspension of cholesterol in water. These results, however, have not been confirmed by subsequent investigations [u] in which attempts to produce generalized changes in the arteries by means of injections of cholesterol have met consistently with failure. Various solutions of cholesterol and various routes of administration have been tried, but the parenteral introduction of cholesterol has not proved itself a method well adapted to the production of lesions in the arteries, perhaps because the doses of cholesterol are necessarily rather small or because the experiments have not been sufficiently protracted. Apparently cholesterol is absorbed rather slowly from the tissues even when it is dissolved in oil. Oily solutions can hardly be injected intravenously in large quantities, and colloidal suspensions of cholesterol in water, when injected into the veins, produce only a relatively slight and often temporary elevation of the blood cholesterol level. The increase occurs chiefly in the free cholesterol in the plasma, while the cholesterol esters remain practically unchanged. The cholesterol is rapidly removed from the blood and is found in the reticulo-endothelial cells. Evidently other lipoids are required to hold the cholesterol in the blood stream for any length of time in stable colloidal solution.

IV. ARTERIAL LESIONS OF EXPERIMENTAL CHOLESTEROL ARTERIOSCLEROSIS IN RABBITS

(*a*) *Gross Appearance and Distribution.*—The descriptions by numerous authors [v] of the gross appearance and distribution of the

(*u*) 38, 46, 69, 70, 123, 140, 167, 189.

(*v*) 4, 5, 20, 45, 66, 100, 131, 147, 148, 152, 153, 162, 164, 166, etc.

arterial lesions which follow cholesterol feeding in rabbits show a general agreement, except when the experiment has been modified by procedures other than simple administration of cholesterol. The lesions of the arteries make their appearance first in the aorta but only after the liver, spleen, bone marrow and suprarenal glands have become loaded with lipoids. In the aorta, the earliest alterations are usually to be found in the arch about the mouths of the vessels arising from it or just above the aortic valve ring. They become visible on the intimal surface as minute yellowish-white opaque flecks which are raised slightly above the surrounding normal intima. At first these areas are not sharply limited but fade off gradually at their borders. However, with increasing size, the tiny thickenings often come to form well demarcated little nodular projections of rounded or irregular outline, which have a curious glistening appearance and a yellow or yellowish-white color. As the original lesions become larger, more and more yellowish opacities make their appearance in other parts of the arch, and they may be found also in the thoracic aorta. In the latter situation, they are more numerous on the posterior wall of the vessel and are usually described as tending to favor the areas about the mouths of the intercostal arteries. However, similar thickenings are often present on the sides or on the anterior aspect of the aortic wall. In some cases the lesions may be confined to the arch and thoracic portion of the aorta,[20] but the abdominal portion is frequently affected also, though to a lesser degree, and here, too, the areas about the mouths of branching arteries are more commonly the sites of lesions than areas elsewhere.

With increase in the dimensions of the individual nodules, which may reach the size of a split pea, adjacent nodules coalesce, forming large irregular plaques, or this may occur widely, especially in the arch of the aorta, so that large areas become involved. A rough, warty, yellowish surface results, and the thickening may be sufficient to cause a significant narrowing of the aortic lumen,[100] though later a dilatation of the vessel occurs. In the thoracic portion of the aorta, the confluence of a series of nodules may form irregular streaks, especially on the posterior surface of the vessel. In the abdominal portion, the irregular coalescence of individual nodules may produce a variegated picture. The most severe changes in the aorta are characterized by the appearance of fusiform dilatations usually in the arch, followed rarely by the formation of true aneurysmal sacs.[89] The elasticity of the vessel is appreciably reduced.[105]

When the lesions in the aorta have become well established, and sometimes sooner, isolated fatty flecks may be found also in its larger branches, perhaps most commonly at points of bifurcation. The innominate, common carotid, subclavian, common iliac and femoral arteries are often the sites of lipoid accumulations, but the renal arteries are

not frequently mentioned. Later, even the smaller branches of the arterial tree may show small nodular lesions. The coronary arteries of the heart are often attacked[w] and to a lesser degree those of the spleen and mesentery. The arteries of the liver and kidney are even less affected, while the cerebral and retinal arteries always remain unchanged.[x]

The main trunk of the pulmonary artery also shows fatty thickenings on the intimal surface soon after the earliest changes are found in the aorta. Small yellowish-white nodules first make their appearance in irregular distribution and later coalesce as in the arch of the aorta, forming a roughened and irregular surface. Isolated plaques may be found also in the large branches of the pulmonary artery and even in its finer ramifications.[y]

(*b*) *Microscopic Appearance of Lesions in the Aorta.*—Numerous descriptions of the microscopic appearance of these lesions in the arteries of rabbits are to be found in the literature,[z] and regarding certain of their more obvious features there is no question. However, the lesions vary to a considerable extent in some respects even in apparently comparable series of experiments. This is evident in a comparison of a number of descriptions of the alterations in the arteries and is even more striking in a comparison of photomicrographs or drawings of the arterial changes. How much of this variation may be due to differences in the rate of development of the lesions with different daily doses of cholesterol or to natural differences between individual rabbits, or how much may depend on unknown and uncontrolled factors, can be a matter only for speculation at present. In any event, differences do exist. In the intima, they are to be found chiefly in the varying abundance of cells relative to the quantity of intercellular material and in the varying proportion between the numbers of the two main kinds of cells which appear. Often the prominent feature in the intima is the accumulation of large, foamy, fat-laden cells, which may compose the bulk of the thickening, while in other experiments the fibroblastic reaction is more pronounced. In still other cases, the intimal thickening is extremely poor in cells and is composed largely of homogeneous intercellular material, which I shall hereafter refer to as "ground substance," recognizing, of course, that it may exist in vivo as a fluid or perhaps in the form of a gel. In the media, the character of the lesions is much more constant, but the extent of the medial involvement varies considerably. Focal necrosis of the smooth muscle of the media followed

(*w*) 81, 84, 184.

(*x*) 11, 12, 162, 164.

(*y*) 20, 29, 45, 99, 100, 135, 179.

(*z*) 4, 5, 20, 34, 45, 66, 76, 80, 89, 100, 126, 131, 135, 147, 148, 152, 153, 166, 169, 189, etc.

by impregnation of these areas with lipoids may be a striking and early occurrence,[a] or the appearance of the medial lesions may be delayed until the lesions in the intima are well developed.

The lesions in the large branches of the aorta and in the smaller arteries have not been studied so thoroughly as those in the aorta itself. However, the lesions, wherever they occur in the arterial tree, seem to be of essentially the same nature. The description of the histologic changes in the arteries will therefore be confined to those occurring in the aorta.

Microscopic alterations in the aorta can sometimes be distinguished a month or more before the lesions become grossly evident, but the site of the earliest deposits of lipoid material is a question about which there has been discussion. In the rabbit, the intimal layer of the aorta consists of a single layer of lining endothelium separated from the internal elastic lamina only by an extremely thin layer of intercellular ground substance in which an occasional spindle-shaped cell may be seen. The thin layer of ground substance first becomes swollen so that it is distinctly thicker than usual. It was in this layer of altered intercellular substance that Anitschkow[b] and others[c] observed the earliest deposits of anisotropic fat as fine droplets or granules, prior to the appearance of any fat-containing cells. Other authors[d] concluded from their observations that the first appearance of lipoids was within cells of the lining endothelium or of the subendothelial layer. The burden of proof seems to rest with the latter group, for they all observed extracellular as well as intracellular fat. While the extracellular lipoid material might have been released from necrotic cells, the possibility that it was first deposited in the intercellular material and later was taken up by cells cannot be excluded. From my own observations[45] I am convinced that anisotropic fatty materials may appear extracellularly in the intima in the altered subendothelial ground substance before any fat-containing cells are to be found. Indeed, the accumulation of lipoids in the greatly swollen subendothelial layer may become very abundant without the appearance of any considerable cellular proliferation. On the other hand, the extracellular lipoids may rapidly be masked by the advent of numerous cells which become laden with fat. It was found also that the inner layers of the media may be the site of the earliest deposits of lipoid materials, as will be described more fully in a subsequent paragraph.

At a slightly later stage, the thickenings of the intima of the aorta nearly always contain a considerable accumulation of cells, among which

(*a*) 20, 45.
(*b*) 4, 5.
(*c*) 126, 145, 164, 189, etc.
(*d*) 20, 99, 100, 166.

large, foamy, fat-laden cells are the most prominent. These foamy cells may sometimes show a palisade arrangement in rows vertical to the plane of the aortic surface,[100] but more frequently they are irregularly arranged and form a little heaped-up clump, which eventually becomes grossly visible as a tiny yellowish-white thickening on the intimal surface. The diameter of the individual cells ranges from 10 to 24 microns,[e] and their outline may be rounded or polygonal or even somewhat flattened. In the smaller cells the cytoplasm forms only a relatively narrow rim about the nucleus, but in the larger ones it is voluminous. It is pale-staining and slightly basophilic, and shows a delicate reticular structure forming a spongy or honeycombed network which gives the cytoplasm a foamy appearance. The nucleus is usually centrally placed, small, round and deeply staining. In larger cells, however, it may be paler-staining or in some cases small and pyknotic, and it may be displaced from its central position. Some of the large cells contain two or three nuclei.[f] It will be convenient to refer to the cells described in this paragraph as "foam cells."

The phagocytic foam cells have attracted special attention because of their exact resemblance in form and apparently also in function to the large fat-holding cells found in the intima of arteriosclerotic human arteries. The foam cells which accumulate in the intima in experimental cholesterol arteriosclerosis seem unquestionably to be macrophages. They resemble the large mononuclear phagocytes elsewhere in the animal body not only in appearance but also in their active phagocytosis of lipoids. Moreover, they take up colloidal dyes just as do the macrophages elsewhere,[70] so that there is really no reasonable doubt as to their identity. It is possible that the macrophages might migrate into the intima from the medial direction, but this possibility is practically excluded by the lack of foam cell accumulations in association with deposits of anisotropic lipoids in the media.[g] There are those who believe that the macrophages enter the intima from the lumen of the artery. However, it appears quite possible that they may arise through the multiplication of wandering mononuclear phagocytes present in the intima prior to the appearance of lipoids. In any event, this is a possibility which has not been excluded.

In the interstices between the foam cells there are found other, much smaller, spindle-shaped or stellate cells with round or oval nuclei. These are morphologically indistinguishable from fibroblasts and are usually identified as such. These cells may be quite prominent in the early stages,[20] but more often they are not very numerous until the lesions become more advanced. The description by some of the

(*e*) 66, 147, 148.
(*f*) 4, 100.
(*g*) 45, 70.

earlier investigators of smooth muscle cells in the intima seems surely to have been due to a mistake in the identification of the spindle-shaped cells or in the recognition of the limits of the media after the internal elastic lamina had disintegrated. Several authors also mention the occasional occurrence in the intima of small mononuclear cells resembling blood lymphocytes.

Even after the appearance of numerous cells in the intima, the homogeneous intercellular ground substance may remain quite conspicuous, and it appears to increase in abundance. Fine strands of fibroglia and delicate elastic fibrils are also found ramifying between the cells of the intimal thickening.

Opinion varies as to the rôle in the reaction played by the lining endothelial cells. Anitschkow [h] believed that they were entirely passive, and stated that they could always be seen forming a single thin layer of flat cells covering the surface of the thickening in the intima. However, this observation has not always been confirmed. Most authors have found many of the intimal cells covering the lesion somewhat swollen and containing fatty granules, while other cells were thin and flat. But they hesitate to call the swollen cells lining endothelium, although the cells are apparently in contact with the blood stream. This hesitancy is perhaps justified because of the frequent difficulty in seeing any endothelial lining in the normal rabbit's aorta and the ease with which it can be wiped off in handling the gross material.

In frozen sections stained for fat, the aortic lesions are seen to contain large amounts of lipoid material. The cytoplasm of the foam cells is packed with innumerable fine granules or droplets which stain with sudan III and which, in unstained sections, show a pale yellow luster. Many of the spindle-shaped cells contain fatty granules scattered in the cytoplasm at either end of the nucleus. The intercellular ground substance contains deposits of finely divided lipoid material or it may be densely and uniformly impregnated with lipoids. Crystals of lipoid material may be seen in the foam cells as well as in the intercellular ground substance of the intima and, in paraffin sections, fusiform lipoid-crystal clefts are frequently seen in both situations.[1] A large proportion of the fatty material is anisotropic. In consideration of this fact and of the other physical properties as well as the staining reactions of the lipoid material, it seems probable that the deposited lipoids consist chiefly of cholesterol and its esters.[4] However, this cannot be stated with certainty since the staining reactions and other criteria commonly employed for the identification of lipoids in histologic sections are subject to certain fallacies, as Kutschera-Aichbergen has shown.[82]

(*h*) 4, 12.
(*i*) 45, 100.

Chemical determinations of the lipoids contained in the arterial lesions of experimental cholesterol arteriosclerosis have apparently never been made.

With the further development of the lesions, the thickness of the intima may reach 126 microns,[j] or it may be even greater than that of the underlying media.[20] The foam cells in the deepest part of the plaque become necrotic, leaving their load of fatty material free in the finely granular débris of their disintegration. At the same time fibroblasts come into prominence. They proliferate extensively in the deeper layers of the intimal thickening and frequently form a compact fibrous layer just beneath the intimal surface. There is a marked increase in fibroglia and elastic fibrils, the latter often showing a connection with the splitting internal elastic lamina. A large number of foam cells still persist, scattered singly and in groups throughout the lesion. An aggregation of foam cells usually surrounds the collection of free lipoid material in the depths of the intimal thickening and adds to its bulk through necrosis. The "pool" of free fatty substance which results tends to become calcified, and the calcification tends to involve also the underlying media, which by this time has usually undergone extensive changes.

In order to appreciate properly the significance of the deposition of lipoids in the media of the aorta, one must return to the earliest microscopic changes in the vessel and follow the process. As has been mentioned, the changes in the media may be the earliest to appear and may remain the most striking. So far as I am aware, there is no record in previous literature of lipoid deposits in the media preceding those in the intima, although Bailey [20] remarked on the extent of the medial lesions in some of his rabbits when the intimal deposits were only beginnning. However, I have observed in rabbits of my own experimental series [45] very conspicuous deposits of anisotropic lipoid material in the media of the arch before any change could be detected in the overlying intima either grossly or microscopically. These deposits were preceded by a peculiar focal necrosis of groups of muscle fibers in the inner third of the media. The affected areas were at first quite small, occupying the space between two adjacent elastic laminae and extending for a short distance in the longitudinal direction. In such areas there were no intact muscle cells, the spaces containing only a very pale-staining, rather cloudy or flocculent ground substance with occasional fragments of cells. The smaller areas of necrosis never showed any lipoid deposits, but in the larger ones the beginning accumulation of fine granules or droplets of anisotropic lipoid became evident. The lipoid droplets were diffusely distributed through the floc-

(*j*) 147, 148.

culent ground substance, and in still larger areas lipoids were even more abundant. The fatty material was almost exclusively extracellular and lay free in the anuclear areas of muscle destruction. Occasional small mononuclear cells contained a few tiny droplets of stainable fat in their cytoplasm. However, the abundant accumulations of fat-laden foam cells, so common in the lesions of the intima, were entirely lacking in the media.

The more severe degrees of medial damage in these animals always had associated with them a more or less marked change in the overlying intima, and this appears always to have been the case in the observations of others. With advancement of the medial lesions, there occurs widespread destruction of the muscle and elastic tissue in the inner third of the media followed by a progressive infiltration of lipoids. The individual areas of anisotropic lipoid deposit finally coalesce so that the inner medial zone comes to consist only of pale-staining structureless ground substance densely impregnated with lipoids and containing only occasional distorted muscle cells and fragments of elastic fibers scattered through it. The internal elastic lamina resists destruction for a long time, but finally it, too, shows breaks in its continuity. This event marks the first appearance of the large foam cells in the media. They can be seen first near the points of rupture of the internal elastic lamina and seem to be wandering outward from the intimal layer.[k] The internal elastic lamina may eventually undergo complete disintegration, and the inner layer of the media, packed with fat-laden foam cells, becomes almost indistinguishable from the intima. The destruction of the media may advance until only its outer third remains intact. This widespread destruction with weakening of the media is evidently the basis for the formation of the aortic dilatations and aneurysms which may occur in the late stages of the process.[89]

A number of investigators[1] have described the early anuclear areas of fatty infiltration in the media, but the fact of the occurrence of the medial lesions has never been given any prominence. They are frequently given mere mention, and it is tacitly implied that they are only the result of "fatty degeneration" of the media secondary to the intimal lesions. It is true that they are commonly associated with lesions in the overlying intima. However, it is clear that they sometimes occur independently, and that the lipoid accumulations are not due to a "fatty degeneration" of the muscle fibers but represent the result of lipoid deposition following injury and necrosis of the media. These facts are of considerable importance in the interpretation of the significance of experimental cholesterol arteriosclerosis.

(*k*) 45, 100.

(*l*) 20, 26, 70, 89, 147, 148, 152, 166, etc.

(*c*) *Changes in Arterial Lesions Following Cessation of Cholesterol Feeding.*—The fate of the lesions in the aorta after the feeding of cholesterol is discontinued is a point on which less information is available. However, several investigators [m] have reported a few experiments in which the lesions were examined at intervals up to six months after the cessation of cholesterol feeding. Anitschkow [11] carried out more extended experiments in which the intervals after the withdrawal of cholesterol from the diet ranged from 101 to 815 days. Similar investigations of the lesions in the coronary arteries of the heart have been reported.[n] In all of these experiments, cholesterol feeding was continued long enough for the lesions in the arteries presumably to be well developed. After the cessation of cholesterol administration, the arterial lesions were found to persist to the end of the longest experiments, but their character was somewhat altered. No appreciable changes could be seen in the aortic lesions within a month,[169] but after longer intervals they showed evidences of transition toward a more fibrous type.

The results of these experiments indicate that the fatty materials in the plaques gradually diminish in quantity, a process which seems to be more rapid in the arch of the aorta than in its more distal parts.[11] The superficial layers of the thickenings come to be composed chiefly of fibrous connective tissue cells interspersed between abundant collagen and elastic fibrils. Lipoids still persist, however, especially in the deeper parts of the plaques, where numerous anisotropic crystals are found lying in necrotic débris. Calcification in these areas is a frequent occurrence. The fat-containing foam cells diminish in size and number and are found mostly about the collection of free fatty material in the depths of the lesion. Muscle fibers are frequently mentioned as occupying a similar situation. Their presence probably indicates disintegration of the internal elastic lamina with medial involvement, but it is impossible to be certain of this, since the condition of the underlying media has not been specifically described. The smallest lesions which remain are composed almost entirely of fine fibrillar connective tissue, but even in these there are found a few fat-containing foam cells or scattered lipoid crystals. Hyalinization of the fibrous tissue has never been described as occurring in the intimal thickenings, even at the end of these prolonged experiments.

It would be interesting to know whether or not the earliest minimal lesions would disappear completely after the cessation of cholesterol feeding.

(*d*) *Mutual Independence of Experimental Cholesterol Arteriosclerosis and Spontaneous Disease of the Arteries of Rabbits.*—Before

(*m*) 80, 131, 152, 169.
(*n*) 81, 184.

proceeding farther, it is necessary to consider the possible rôle of spontaneous arterial disease in the development of the arterial lesions described in the foregoing pages. This is especially important in the case of the rabbit, which is the animal that has been used in the great majority of experiments with cholesterol feeding and in which spontaneous disease of the arteries is well known to occur.

Descriptions of spontaneous changes in the arteries of rabbits [o] are confined almost exclusively to one type of lesion in which alterations are found chiefly in the media of the aorta. The lesions occur in localized areas in the middle of the medial coat or nearer its inner margin. The muscle cells become necrotic and disappear and the elastic fibers, while remaining intact, lose their undulations and fall together in parallel rows. Calcification in the affected areas is a common end-result. Stainable fat, if present at all, occurs in only the most minute amounts within degenerating muscle cells or in scattered wandering cells, and it is not anisotropic. The intima is affected secondarily, showing inconspicuous alterations in the lining endothelium and some swelling of the subendothelial ground substance. The frequency with which this type of spontaneous lesion occurs gives some indication of the susceptibility of the rabbit to arterial injury, but the lesion itself need not be considered further, since it is perfectly clear that it is quite different from the lesions produced by the administration of cholesterol-rich diets. When spontaneous medial lesions are present in a rabbit which has been subjected to cholesterol feeding, they can still be recognized with the greatest ease and distinctly differentiated from the lesions resulting from the feeding.[36] I have thoroughly convinced myself of the truth of this statement in my own experiments.

Nuzum and his co-workers [113] recently drew attention to another type of arterial lesion which they encountered in rabbits as a spontaneous development. The lesions which they described are indistinguishable from those which follow the administration of cholesterol-rich diets. In the examination of 190 rabbits, lesions of this type were found in the aortas of 6 of them. All of the animals were between 2 and 3 years old, and most of them had been used in the standardization of insulin or as controls in various experiments. The importance of the observation cannot be denied, but it loses much of its significance in the face of the evidence accumulated by a number of other investigators.

So far as I am aware, the only report of a similar finding is that of Ophüls,[116] who found lesions of the type in question in the aorta of 1 rabbit. The animal had just been received in the laboratory and its age was unknown, but it was described as a large, healthy, well developed, female rabbit. Ophüls thought the observation sufficiently

(*o*) 23, 36, 43, 61, 87, 95, 96, 101, 113, 118, 171.

unique to warrant a separate publication. On the other hand, similar lesions have never been encountered among the several thousands of rabbits examined by other investigators[p] in search of spontaneous arterial disease. Failure to find the lesions could hardly be the result of failure to examine the arteries microscopically, for in most instances microscopic examinations were made. In view of these facts, it seems hardly credible that spontaneous arterial lesions of this type, heretofore so excessively rare, should suddenly crop out in a relatively small series of 190 animals without some definite cause. It is, of course, impossible to know exactly what the cause was, but it seems that the use of some of the animals for apparently innocent experimental purposes may not have been entirely without effect. Dietary factors acting over a long period of time may have been responsible. This is possible since the rabbits were all old ones, and the suggestion seems all the more plausible since it is known that during the prolonged administration of diets containing very small quantities of cholesterol, lesions of the type in question develop in the aorta.[7] The same explanation may well apply in the case of the rabbit described by Ophüls, the previous diet of which was entirely unknown. In any event, confirmatory reports from other laboratories in which the routine diets for rabbits is different would be requisite to a firm conviction regarding the strict spontaneity of the aortic lesions under discussion.

Whatever may be one's opinion concerning the cause of these "spontaneous" changes, it still seems certain that the fatty arterial lesions observed in the numerous experiments on rabbits were produced by the experimental procedures employed, and this for three reasons: 1. In no series of experiments have lesions of the type in question been found in control animals. 2. Barring accidents, the arterial lesions can be produced without fail in young rabbits by the use of sufficient doses of cholesterol administered in a suitable way. 3. Very young rabbits are just as susceptible to the effects of cholesterol feeding as adult animals. This fact is illustrated in the experiments of Starokadomsky,[147] who was able to produce typical fatty lesions of the aorta in all of 6 rabbits which at the end of the experiment were only 80 days old. For these reasons it is concluded that the arterial lesions of experimental cholesterol arteriosclerosis in rabbits may safely be considered as one of the results of the feeding of cholesterol-rich diets.

V. EFFECTS OF CHOLESTEROL-RICH DIETS ON THE ARTERIES OF ANIMALS OTHER THAN RABBITS

The method of cholesterol feeding has now been applied to a variety of experimental animals in attempts to produce fatty lesions in the arteries, but for the most part without success. It seems probable that

(*p*) 23, 36, 43, 61, 87, 95, 96, 101, 118, 171.

experimental arteriosclerosis can be produced in guinea-pigs by cholesterol feeding but only with considerably greater difficulty than in rabbits. Fatty lesions in the aorta and sometimes in other arteries have been observed in pigeons, chickens, a parrot, mice, rats and goats following prolonged periods of cholesterol feeding. All of the experiments on the latter animals, however, are open to serious objections. The possibility that the arterial lesions had arisen spontaneously has been almost completely ignored, and in many instances control observations on the arteries of untreated animals are entirely lacking. Nevertheless, these experiments are frequently cited as evidence that experimental cholesterol arteriosclerosis can be produced in a wide variety of animals. In certain other animals the failure of cholesterol feeding to affect the arteries is admitted by every one; all attempts to produce arterial lesions in cats, dogs, foxes and monkeys by feeding cholesterol or cholesterol-rich diets have been uniformly without success.

The experiments on the various animals mentioned will be outlined as briefly as possible. However, some of the experiments must be described in greater detail than they deserve, merely to demonstrate their inadequacy as a basis for the statements concerning them which constantly recur in the literature.

(*a*) *Birds.*—The literature dealing with the results of cholesterol feeding in birds is extremely unsatisfactory though frequently quoted. The experiments are few in number and poorly controlled or not controlled at all, so that the results are far from convincing. So far as I can determine, there are recorded in the literature experiments on 7 pigeons, 20 chickens and 1 parrot. These experiments will be considered in order.

Pigeons: Anitschkow [12] has referred to three investigators as authority for his statement that lipoid infiltration of the arterial walls can be produced in pigeons by feeding them with egg yolks, hydrous wool fat or cholesterol. Of these investigators, only Yamaguchi [187] reported any experiments on pigeons; his pigeons were fed with egg yolks or hydrous wool fat. The experiments were described in Japanese in 1922, and the only available source of information concerning them (and the source to which the European investigators refer) is Kawamura's monograph,[70] in which Yamaguchi's report of his experiments with pigeons and chickens is quoted at length. Apparently Yamaguchi fed 4 pigeons with 1 or 2 egg yolks daily for periods of from 562 to 603 days. Three other pigeons were fed quantities of hydrous wool fat for even longer periods. It is not clear what lesions were found in the arteries at the termination of the experiments, for Kawamura has described together the results obtained by Yamaguchi in both pigeons and chickens. Some parts of the description refer specifically to chickens, some parts refer to pigeons, but other parts may refer to either or both. However, this much seems

to be clear. The 4 pigeons fed with egg yolks showed gross "atherosclerosis" of the aorta; in 1 of these there was "a general thickening of the first part of the aorta." The 3 pigeons fed with hydrous wool fat showed no gross lesions but evidently some thickening of the intima was seen microscopically. In describing the microscopic changes in the arteries, Kawamura failed to distinguish between the lesions found in pigeons and those in chickens, but one gathers that the pigeons' aortas probably showed some deposits of lipoids in the intimal thickenings and in the inner layers of the media.

Whatever may have been the exact nature of the lesions observed in the arteries of the 7 pigeons, there is absolutely no proof that the lesions were not spontaneous nor is there any proof that they had developed as the result of feeding the pigeons egg yolks or hydrous wool fat. No control animals were mentioned, and Kawamura himself seemed to doubt that the cholesterol in the diet had produced the changes in the arteries. In another part of Kawamura's monograph are recorded Yamaguchi's observations on the distribution of cholesterol in the organs of a variety of species of birds including pigeons, all presumably normal, but there is no mention of the condition of the arteries in the pigeons or in any of the other birds excepting chickens. Spontaneous arteriosclerosis, however, does occur in pigeons, as described by Fox.[48]

Although cholesterol-rich diets may be capable of producing lesions in the arteries of pigeons, it is difficult to understand how any one can regard this possibility as an established fact on the basis of the nebulous information outlined in the preceding paragraphs. One can conclude only that the feeding of cholesterol-rich diets has not been shown to have any effect whatever on the arteries of pigeons.

Chickens: In conjunction with his experiments on pigeons, Yamaguchi [187] also experimented on 11 chickens, which he fed daily with egg yolks or hydrous wool fat for periods up to 807 days. Two of the 5 chickens which had been fed egg yolks showed "atherosclerosis" of the aorta, and in 1 of the 2 there were distinct changes in the intima of the large branches of the aorta. The arteries of the 6 chickens which had been fed with hydrous wool fat showed no gross lesions. In commenting on Yamaguchi's experiments with pigeons, I have pointed out the difficulty of obtaining from Kawamura's résumé of the experiments any exact information concerning the lesions in the arteries. The same difficulties are encountered in the case of the experiments on chickens. Again no control animals were mentioned by Kawamura, although he remarked in another part of his monograph that Yamaguchi had observed arteriosclerosis in apparently normal chickens. Uchiyama,[160] who presumably had access to Yamaguchi's Japanese papers, stated that the latter had found spontaneous "atherosclerosis" of the

aorta in about 75 per cent of all the untreated chickens examined and that he had not observed any increase in the arterial changes in chickens fed egg yolks.

The frequent occurrence of spontaneous arteriosclerosis in chickens is well recognized. The spontaneous lesions in the arteries have been described in detail by Uchiyama,[160] who found that they were almost always present in the aorta by the end of the second year of life. The lesions consisted of localized accumulations of lipoids in the intima and inner layers of the media with fibrous proliferation in the former and sometimes with the formation of atheromas. Necrosis and calcification of the media were found in more diffuse distribution, especially in the distal half of the aorta, which in chickens has the structure of a muscular artery. Uchiyama also carried out feeding experiments on 9 chickens which were fed daily 0.3 Gm. of cholesterol in oil for periods of from 12 to 420 days. In some of these chickens he found arterial lesions of exactly the same character and distribution as those occurring spontaneously in the control animals, but he believed that the intimal changes in the chickens of the experimental group were more advanced. Confirmation of this opinion by clearcut and carefully controlled experiments will be necessary to any firm conviction regarding the ability of cholesterol feeding to affect the arteries of chickens.

Parrots: Wolkoff [183] has described the lesions found in the arteries of a 40 year old parrot which had been fed egg yolks almost daily during the last 3 years of life. This apparently is the parrot referred to by Anitschkow.[9] Both Wolkoff and Anitschkow seemed to think that the feeding of egg yolks had had some bearing on the production of the arterial lesions, but this can be nothing more than an opinion since no control animals were examined. Identical spontaneous arteriosclerotic changes have been found by a number of different investigators [q] in parrots of comparable age or younger given diets containing no foods especially rich in cholesterol. There is no reason, therefore, to believe that the lesions in the arteries of the parrot described by Wolkoff were in any way dependent on the feeding of egg yolks, and it seems altogether likely that they were of spontaneous origin.

(*b*) *Mammals.*—Guinea-Pigs: As early as 1913 Chalatow [30] had tried the effect of lipoid diets on several guinea-pigs. Anisotropic lipoid deposits were found in 1 animal, chiefly in the liver and spleen, but no mention was made of any changes in the arteries. However, in 1915 Bailey [19] succeeded in producing fatty lesions in the aortas of guinea-pigs by feeding from 0.1 to 0.5 Gm. of cholesterol in oil daily for periods up to 72 days. No alterations were visible to the naked eye, but microscopically he found "small patches of fatty infiltration in the intima

(*q*) 22, 48, 60, 111, 117.

and upper media. The characteristic proliferation and subsequent degeneration seen in the rabbit were entirely lacking." He felt that the feeding period was too short to exclude the possibility that proliferative changes might occur.

Anitschkow [6] repeated the experiment some years later on 7 guinea-pigs which he fed from ⅓ to 1 egg yolk daily. The longest experiment lasted 183 days. In the 3 animals which were fed for more than 62 days, he was able to make out minimal gross changes in the form of isolated tiny yellowish streaks in the first part of the arch of the aorta. Microscopically the lesions consisted of a diffuse infiltration of lipoid material in the subendothelial layer of the intima and in the inner layers of the media. Drawings show clearly that the fatty deposits in the media were located in anuclear areas from which the muscle cells had disappeared. The more advanced lesions showed cellular accumulations in the intima consisting of cells like small blood lymphocytes and of large fat-laden foam cells. The internal elastic lamina was sometimes split and connected with fine elastic fibrils in the thickened intimal layer. In some cases it showed breaks near which cells were found in the media similar to those seen in the intima.

The briefer reports of several other investigators [r] lend corroboration to these observations.

Unfortunately, no control animals appear to have been studied in connection with any of these experiments, and this fact introduces some doubt as to the status of the arterial lesions described. I have been unable to find much information concerning the occurrence of spontaneous disease of the arteries of guinea-pigs. Krause [78] and Löwenthal [94] did not mention guinea-pigs in their reviews dealing with spontaneous arterial diseases in animals. However, Weinberg [171] stated that he had examined the aortas of 236 guinea-pigs without encountering arterial lesions of any kind. Saphir [130] also reported the absence of lesions in the aortas of 30 apparently normal guinea-pigs. In view of these completely negative observations in normal animals and in consideration of the fact that arterial lesions were found in a fairly large proportion of the experimental animals, it seems that the changes in the arteries of guinea-pigs attributed to cholesterol feeding may be tentatively accepted.

Mice: The first reports of fatty changes in the arteries of cholesterol-fed mice were made by Löwenthal [s] in 1925. He experimented with white mice, which he fed daily 0.015 Gm. of cholesterol in oil. In addition to being fed cholesterol, some of the mice were given egg-white and others were castrated. Only 1 of the 4 mice which received no treatment other than cholesterol feeding presented any fatty deposit in the arteries. It had been fed for about 4½ months, and at the end

(r) 70, 135, 156.
(s) 92, 93.

of that time it showed a single microscopic area of fat deposit just above the aortic valve ring. The subendothelial layer of the intima contained fatty granules, and a few small cells also contained lipoid material. Among the 22 mice fed cholesterol and egg-white and in the emasculated group, 12 animals had microscopic fatty lesions in the aorta, characterized by an accumulation of finely divided lipoid material in the subendothelial layer, part of which was contained in spindle-shaped cells. Some of the aortas also showed an infiltration of the inner layers of the media with fatty granules.

Yuasa [188] fed mice daily 0.03 Gm. of cholesterol in 0.2 Gm. of lard for from 80 to 158 days. Of the 14 mice, 6 showed fatty lesions in the aorta, the minimum time for their development being 126 days. The changes in the aorta were not described in great detail. They consisted essentially of an accumulation of anisotropic lipoid droplets in the intima and neighboring media with some cellular proliferation in the former.

Wolkoff [185] found similar microscopic aortic lesions in 3 of 11 mice which had been fed cholesterol-rich diets. In one animal, feeding had been in progress for 182 days, and in the other 2, for 425 days.

Although Wolkoff reported 6 control animals in which there were no aortic lesions, nevertheless, mice appear to be subject to the development of spontaneous arterial changes which may be identical with those found after cholesterol feeding. Löwenthal [94] found such microscopic changes in 9 mice among about 85 which he examined. In his experiments with cholesterol feeding [93] he found arterial lesions in 13 of 26 mice, but 12 of the 13 had been castrated or fed egg-white in addition to being fed cholesterol and, moreover, 3 of the 6 control animals also showed arterial lesions. Yuasa apparently made no observations on the arteries of control animals.

On the basis of such evidence, one seems hardly justified in concluding that cholesterol feeding alone is capable of producing arterial lesions in mice. Indeed, lack of adequate controls, together with the fact that similar arterial lesions occur not infrequently in untreated animals, renders these experiments practically worthless for the purposes of this paper.

Rats: In his earlier experiments Chalatow [31] was unable to produce deposits of anisotropic fat in the organs of white rats without the addition of phosphorus or fatty acids to the cholesterol feeding. Although he then found abundant deposits of anisotropic lipoids in the spleen and isotropic fat in the liver and suprarenal glands, he found no changes in the arteries. In a later series of 24 experiments [32] he obtained similar results. No arterial changes were produced even by heavy and prolonged feeding of cholesterol except in 1 animal. This rat had received a total of about 35 Gm. of cholesterol in oil within 4½ months. Fatty acids and phosphorus were also administered in varying quantities.

The aorta of this animal showed intimal thickenings in which were found deposits of finely divided anisotropic fat, partly contained within large phagocytic cells and partly dispersed through an abundant homogeneous intercellular material.

In Kon's experiments with rats fed various lipoid diets [76] arterial changes were completely lacking.

In consideration of the great rarity of arterial lesions in the experiments of previous investigators, Yuasa's results [188] seem rather surprising. His rats were given a daily dose of 0.1 Gm. of cholesterol in from 1 to 2 Gm. of lard. Five of the 9 rats showed slight deposits of anisotropic fat in their aortas, the earliest change being found after 96 days of feeding. The lesions consisted of an accumulation of numerous droplets or granules of anisotropic lipoids in the intima and inner layers of the media.

In Yuasa's account of his experiments with rats no reference was made to any observations on the arteries of control animals. Chalatow described 2 apparently normal rats as controls for his 24 experiments with cholesterol feeding, etc., but unfortunately he failed to mention the condition of the arteries of the control animals. I have been unable to find any independent information concerning the occurrence or frequency of spontaneous arteriosclerosis in rats. In the complete lack of such information from any source, and in view of the rarity of arterial lesions in rats following cholesterol feeding, one is forced to exclude these experiments from further consideration.

Goats: So far as I am aware, Chalatow's observations on goats fed cholesterol-rich diets [33] are the only ones recorded in the literature. In his experiments, 3 goats were fed from 5 to 15 egg yolks daily for from 2½ to 5 months. The goat which had been fed for the longest time, which had received the greatest total number of egg yolks and in which the blood cholesterol reached the highest level, showed no lesions in the arteries at the termination of the experiment. In 1 of the other goats, there was found a single small fleck of fat in the intima of the arch of the aorta. The aorta of the third goat showed many small flecks of fat in the intima, which were grouped mostly about the mouths of branching vessels. No control observations on the arteries of untreated goats were recorded by Chalatow, so that there is really no evidence to show that the administration of egg yolks was responsible for the development of the arterial lesions.

The occurrence of spontaneous arteriosclerosis in goats is well recognized.[78] It usually takes the form of a calcification of the media of the aorta, but the possibility that lipoid deposits in the intima may occur as a spontaneous development has not been excluded, for this possibility has apparently not been investigated. With these facts in view, it is obvious that Chalatow's experiments, though frequently cited, cannot

be accepted as a demonstration of the ability of cholesterol-rich diets to produce lesions in the arteries of goats.

Cats: Yuasa [188] carried out experiments on 8 cats which he fed daily with a mixture containing meat extract, lard and 1 Gm. of cholesterol. The longest experiment lasted 120 days, but in none of the animals did he find any alterations in the arteries.

The experiments of Cirio [35] were more protracted. He fed 5 cats cholesterol and cholesterol-rich substances for long periods of time. The longest experiment lasted 394 days, during which time the animal was given over 300 Gm. of cholesterol and nearly 40 Kg. of brain tissue. However, no arterial changes were found in this cat or in any of the others.

Dogs: The experimental results of Adler [1] have been cited as evidence that fatty changes in the arteries may follow cholesterol feeding in dogs. However, these results cannot be considered as significant, for Adler himself in a subsequent publication [2] stated that he had observed, in the interval, similar fatty changes in the arteries of normal untreated dogs. He felt that no importance could be attached to his earlier observations of fatty striae in the aortas of dogs which had been fed cholesterol.

Tsunoda and Umehara [157] briefly reported their failure to produce any changes in the arteries of dogs by feeding them for from 1 to 2 years on large quantities of brain tissue. In Kawamura's experiments on dogs [70] the arteries were found to be normal after the prolonged feeding of cholesterol or egg yolks.

Pfleiderer [121] experimented with 3 dogs, each of which was fed 426 Gm. of cholesterol in oil in the course of 496 days. The dogs were also given considerable quantities of irradiated ergosterol, but no lesions were to be found in their arteries at the end of the experiments.

Anitschkow [9] fed a 6 month old dog from 4 to 5 Gm. of cholesterol in oil daily for 14 months. In addition, he produced marked stenosis of the common bile duct by means of ligatures and added to the cholesterol intake by administering bile from another dog with a biliary fistula. He also removed both ovaries and the spleen. By these combined procedures, the blood cholesterol content was raised to about twice its normal value. Nevertheless, no fatty deposits could be demonstrated in the aorta or in the valves of the heart.

Foxes: Yuasa [188] fed 2 young foxes raw meat and lard for 3 months. In addition, 1 of the foxes was given a daily dose of 1 Gm. of cholesterol dissolved in the ration of lard. At the end of the experiment, however, no lesions were found in the arteries of either fox.

Monkeys: The only recorded experiments on monkeys are those of Kawamura,[70] which seem to be of the utmost importance. He used

3 monkeys of the species Macacus fuscatus. One was fed 2 egg yolks daily for 150 days. The other 2 were fed quantities of hydrous wool fat. One of these received a total of 1,350 Gm. of hydrous wool fat in 301 days, and the other, 1,650 Gm. of the same substance in 303 days. At the termination of the experiments, no changes in the aorta could be distinguished in any of the monkeys. The liver, spleen and bone marrow contained only insignificant amounts of anisotropic lipoids or else none at all, while the suprarenal glands contained about the same amounts of lipoids as were found in normal control animals.

From the data which have been presented in the foregoing pages, the following conclusions may be drawn: It seems highly probable that experimental cholesterol arteriosclerosis can be produced in guinea-pigs by means of prolonged feeding with cholesterol-rich diets. There is no satisfactory experimental evidence to substantiate the current idea that cholesterol-rich diets can produce lesions in the arteries of pigeons, chickens, parrots, mice, rats or goats. All experiments recorded up to the present time indicate the inability of intensive and prolonged cholesterol feeding to affect in any way the arteries of cats, dogs, foxes or monkeys.

VI. INFLUENCE OF VARIOUS FACTORS ON THE DEVELOPMENT OF EXPERIMENTAL CHOLESTEROL ARTERIOSCLEROSIS

Under this heading, only the experiments on rabbits and guinea-pigs need be considered, for these two species are the only ones in which unequivocal cholesterol arteriosclerosis has been produced experimentally. Of the two species, rabbits have been used much more commonly in experiments with cholesterol feeding, and consequently most of the information available for analysis is concerned with experimental cholesterol arteriosclerosis in rabbits. However, the meager information which has arisen from experiments with guinea-pigs will also be presented. In addition, references will be made to experimental data obtained from the study of other animals whenever such information appears to be pertinent to the subject or of interest for purposes of comparison.

(*a*) *Cholesterol in the Diet.*—There is little remaining doubt that the feeding of cholesterol is one essential procedure in the experimental production of the arterial lesions in rabbits which have been described in a previous section of this paper (section IV). The lesions in the arteries produced by feeding pure cholesterol not dissolved in oil are indistinguishable from those which follow the administration of cholesterol in oil or of diets containing cholesterol-rich foods such as egg yolks. Oils which have been used as solvents and other neutral fats and fatty acids do not have similar effects in control experiments. Wesselkin's experi-

ments [t] indicate that lecithin is not responsible.[u] Indeed, arterial lesions of the type in question have been produced in rabbits only when the administration of cholesterol or of substances containing considerable amounts of cholesterol was part of the experimental procedure. This is the justification for the application of the term "experimental cholesterol arteriosclerosis" to the arterial lesions under consideration. Of course, the possibility still remains that some potent contaminant of supposedly pure cholesterol is also an essential causative or contributory factor, and there are good reasons for believing that lipoids are deposited in the arteries only after the latter have been injured by the action of an unknown damaging agent. Nevertheless, it seems certain that cholesterol administration is one etiologic factor in the production of the experimental arterial lesions in rabbits; that it is the only factor remains open to question.

In guinea-pigs, the effect of adding solid cholesterol to the diet has not been tried. However, arterial lesions have been produced by feeding cholesterol dissolved in oil,[19] and in all other successful experiments with guinea-pigs cholesterol-rich diets were employed. Thus, it seems altogether likely that the etiologic rôle of cholesterol feeding in the development of the fatty arterial lesions is the same in guinea-pigs as in rabbits.

In connection with the feeding of cholesterol the analysis can be carried a little farther. It is obvious that a considerable total quantity of cholesterol must be administered (at least from 10 to 20 Gm. in the rabbit) and that a certain length of time must elapse before any alterations can be detected in the arteries. In general, it is true that large daily doses of cholesterol produce arterial changes more rapidly than do small doses, but the rapidity of the development of the lesions is dependent also on the way in which cholesterol is administered. However, even when cholesterol is given in the most favorable form and in large daily doses to rabbits, microscopic changes in the arteries appear only after from 25 to 30 days of feeding. About twice that length of time is usually necessary for the production of gross lesions. The importance of the factor of time is illustrated by an experiment of McMeans and Klotz.[100] They gave a rabbit daily feedings of cholesterol

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(*t*) 178, 179.

(*u*) Kanócz and László [68] claimed that lecithin has an effect antagonistic to that of cholesterol. The experiments which they offered as a demonstration of this effect were carried out on only 4 rabbits, and the results were ruined by the chance occurrence of calcified spontaneous lesions of the aorta in the 2 animals which had been fed cholesterol without the addition of lecithin. The true character of these arterial lesions is perfectly obvious from the illustrations in their paper, but the authors apparently failed to recognize the spontaneous nature of the calcified lesions in the media, and cholesterol feeding was held responsible. The conclusions drawn on the basis of these experiments cannot be accepted without verification.

in the form of an emulsion with sodium oleate, a preparation which they found to be even more favorable to the production of arterial lesions than solutions of cholesterol in oil. The experiment was continued for 19 days, and although the quantity of cholesterol administered reached the large total of 75 Gm., no gross or microscopic fatty deposits could be found in the arteries. Thus it is clear that the simple administration of cholesterol even in a favorable form does not begin to show its effects on the arteries of rabbits until at least 20 days have passed. So far as I am aware, no trace of lipoid deposits in the arteries of rabbits has ever been observed within a time less than 20 days from the commencement of cholesterol feeding. Even the production of lipoid deposits in less than 25 days is most exceptional, being reported only by Zinserling.[189] This period represents the time necessary when no procedure other than cholesterol administration is employed, and it serves as a useful criterion in estimating the effects of other procedures which may be combined with the administration of cholesterol. Beyond this minimum limit, the factor of time gradually diminishes in importance, and the first appearance or the severity of the lesions comes to depend rather on the total quantity of cholesterol and on the way in which it is given.

No matter in what form cholesterol is fed, it seems to be capable of producing experimental arteriosclerosis in rabbits eventually, if its quantity is sufficient. However, some forms of administration bring results more quickly than others even when the daily dose of cholesterol is the same. The most outstanding difference is to be noted between the feeding of cholesterol in dry powdered form and its administration in oily solutions or in natural emulsions (e. g., egg yolks or hydrous wool fat). To illustrate this point the experience of Wacker and Hueck [166] may be quoted again. They found arterial lesions in their rabbits only after a period of 5 months when about 175 Gm. of powdered cholesterol had been fed. Knack [74] fed a rabbit 382.5 Gm. of solid cholesterol in 85 days and failed to produce any recognizable lesions in the arteries. This is in marked contrast with the earlier appearance of arterial lesions produced by the feeding of much smaller quantities of cholesterol dissolved in oil or contained in egg yolks. Knack [74] and Sternberg [151] pointed out this difference years ago, and their observation has since been reiterated by others, but some investigators still seem to be unaware that the feeding of solid cholesterol is not comparable with its administration in oily solutions or in association with other lipoids as it occurs in the cholesterol-rich foods from natural sources. Failure to appreciate this fact has led to some anomalous conclusions.

The exact reason for this difference is not entirely clear, but the most obvious and simple explanation is that solid cholesterol is not so readily absorbed from the alimentary tract as is cholesterol in oily solutions or in fatty emulsions. The fats not only aid in absorption through their

action as solvents but are themselves absorbed and appear in the blood stream with the cholesterol. It is believed that they serve to hold the cholesterol there by combining with it, partly by the formation of esters and partly in a physico-chemical way.[v]

It is evident that the kind of experimental animal used is a factor of importance. Experimental cholesterol arteriosclerosis can be produced in rabbits with relative ease, but it is a matter of greater difficulty in guinea-pigs.[w] On the other hand, even the most persistent efforts to produce arterial changes in cats, dogs or monkeys by means of cholesterol feeding have met with complete failure. These differences clearly depend, at least in part, on the facility with which the various animals handle exogenous cholesterol. The degree of susceptibility of their arteries to damage probably also plays a rôle.

(*b*) *Hypercholesteremia.*—Interest in the chemistry of the blood of cholesterol-fed animals has centered naturally in the fluctuations of the blood cholesterol and lipoids, and unfortunately other chemical problems have been almost completely neglected. However, the results of studies on the lipoids of the blood are of considerable interest and are of great importance to the purpose of this paper because of the emphasis which has been laid on hypercholesteremia as an etiologic factor both in experimental and in human arteriosclerosis.

The effect of cholesterol feeding on the cholesterol content of the blood varies in different species of animals. Certain carnivores seem to be adequately equipped for the disposal of exogenous cholesterol through utilization, storage or excretion. Thus, dogs respond to single doses of cholesterol dissolved in oil with only a moderate and transient increase in the blood cholesterol content,[x] an increase which is often no greater than that which may occur when the same amount of oil is fed without cholesterol.[y] The continued feeding of cholesterol seems likewise to be incapable of producing any very striking sustained rise in the blood cholesterol level. Grigaut and L'Huillier [56] found a moderate increase in the cholesterol content of the blood of 2 dogs which were receiving daily feedings of cholesterol and butter, but others have observed no significant hypercholesteremia under similar conditions.[z] The few investigations of this nature which have been carried out in cats [a] have given results similar to those obtained in dogs. In rabbits, on the contrary, the mechanism for handling exogenous cholesterol and especially for its excretion is notably deficient, so that a quite enormous increase in the cholesterol content of the blood can be

(*v*) 74, 161, 164.
(*w*) 19, 70.
(*x*) 14, 21, 86, 123, 144.
(*y*) 14, 86.
(*z*) 144, 172, 177.
(*a*) 53, 177

produced by the feeding of cholesterol [b] or of egg yolks.[67] Similar results have been obtained in guinea-pigs.[177]

In rabbits, a course of cholesterol feeding such as is commonly employed for the production of arterial lesions usually results in a very great increase in the blood cholesterol content. Values of from 500 to 1,500 mg. per hundred cubic centimeters are not infrequently reported,[c] and Rohrschneider [126] stated that he had found maximum values ranging from 25 to 50 times the normal as a regular occurrence in rabbits fed for long periods with cholesterol dissolved in oil. The highest figure which he recorded is 2,584 mg. per hundred cubic centimeters. Such high levels, however, are reached only after cholesterol feeding has been continued for some time. With daily feedings of 0.5 Gm. of cholesterol in oil, a few days elapse before the blood cholesterol begins to rise significantly; then it increases rapidly so that at the end of 3 weeks it has risen to 3 or 4 times its original value. The rise is slightly irregular but continuous, and the high maxima mentioned are found after from 3 to 5 months of feeding. The blood cholesterol then drops gradually in spite of continued feeding but does not return nearly to normal.[d] All of this concerns the total cholesterol content of the blood. Other investigations have shown that the greater part of the increase is due to a rise in cholesterol esters, while the augmentation of free cholesterol is not so striking.[e] This is the case even when pure cholesterol without oil is added to the ordinary diet.[167] In addition to the hypercholesteremia there is a parallel increase in neutral fats and in other lipoids so that a severe lipemia results.[f] This shows itself in a milky clouding of the plasma which usually appears within 45 days after the commencement of feeding,[154] but there may be a considerable rise in the total lipoids before distinct clouding becomes visible.[62]

Although it is probable that hypercholesteremia of extreme degree commonly exists in the rabbit during the course of the usual cholesterol feeding experiment in which arterial lesions are produced, it does not necessarily follow that the hypercholesteremia is responsible for the development of the changes in the arteries. The same might be said of the great increase of total lipoids in the blood. Consequently it is important to know whether experimental cholesterol arteriosclerosis can develop without any increase in the blood cholesterol above its normal limit. It seems doubtful that this is possible, but it is clear that excessive hypercholesteremia is not necessary. The lesions in the

(*b*) 38, 49, 126, 137, 154, 155, 161, 167.

(*c*) 126, 154, 155, 167.

(*d*) 126, 137, 154.

(*e*) 62, 161, 167.

(*f*) 62, 116a, 161.

arteries may occur in the presence of a moderate or even a slight increase in the cholesterol content of the blood, as is shown by the following examples.

Prolonged experiments were carried out by Anitschkow [7] on several rabbits which he fed very small quantities of egg yolk in milk. During the experiments a number of chemical examinations of the blood revealed only a slight increase in cholesterol content. At the end of the feeding period he found definite fatty lesions in the aorta. In the experiments of Clarkson and Newburgh [36] and of Deicke [38] certain rabbits, unlike the majority, failed to show more than a slight hypercholesteremia following cholesterol feeding, yet typical fatty lesions were found in their arteries. Certain other rabbits showed a well marked increase of the blood cholesterol, but no arterial lesions were found in them at the end of the experiments. These results led the latter investigators to question the view that the development of the arterial changes is dependent on hypercholesteremia. However, it has never been satisfactorily demonstrated that experimental cholesterol arteriosclerosis can develop in the absence of augmentation of the cholesterol content of the blood. It seems highly probable, and it will be assumed hereafter in this paper, that hypercholesteremia of some degree is an essential factor in the development of experimental cholesterol arteriosclerosis in rabbits.

Now, even if this conclusion is accepted as true, the possibility still remains that hypercholesteremia is not the only essential factor in the development of the arterial lesions of experimental cholesterol arteriosclerosis. It is possible that hypercholesteremia is quite impotent to affect the arteries without the concomitant action of other influences. Indeed, evidence is available to suggest that this is actually the case. Several investigators [g] have recorded observations which show clearly that marked hypercholesteremia may develop during prolonged cholesterol feeding experiments without producing any changes in the arteries within the experimental period, which was greater than 3 months in a number of instances. This point, though not frequently remarked on, is emphasized by Clarkson and Newburgh [36] and by Deicke.[38] If the experiments had been continued for a longer time, arterial lesions probably would have appeared eventually, but the fact remains that at the termination of the experiments marked hypercholesteremia was present and probably had been present for a considerable period of time, and yet no lesions whatever were found in the arteries. This suggests that hypercholesteremia of itself is not solely responsible for the development of experimental cholesterol arteriosclerosis in rabbits. Some additional factor is probably necessary.

(*g*) 36, 38, 116a, 158, 159, 167.

In brief, it seems probable that hypercholesteremia can exist without the development of experimental cholesterol arteriosclerosis, but the latter cannot develop without the existence of hypercholesteremia.

(*c*) *Protein in the Diet.*—There is a general impression that the feeding of protein, especially animal protein, with cholesterol enhances the effect of the latter in producing arterial lesions.[h] This idea was based originally on calculations and comparisons of the cholesterol and protein contents of diets used by different investigators to produce arterial lesions in rabbits. Although the impression may be correct, there is still hardly any direct experimental evidence to support it. Löwenthal's experiments on mice [93] are usually quoted in this connection but, as I have pointed out, there are certain objections to these and other experiments on mice which render them far from convincing.

Newburgh and Clarkson [110] produced fatty lesions in the arteries of rabbits by feeding them diets which contained a rather large proportion of meat, and they concluded that the protein was responsible. In a subsequent publication [36] they reported that the feeding of the meat diet resulted in an increase in blood cholesterol, but that the amount of cholesterol contained in the diet, when fed alone, was insufficient to cause arterial lesions or even to raise the blood cholesterol level. They thought, therefore, that the elevation of the blood cholesterol level in the rabbits on the meat diet must have been due to a metabolic disturbance produced by the excess of protein, and that it was not related to the cholesterol content of the meat. Unfortunately, these conclusions are invalidated by the fact that these investigators in their experiments with the feeding of pure cholesterol administered it in the form of a dry powder so that it probably was not absorbed nearly so completely as was its equivalent in the meat diet. Other lipoids were undoubtedly present in the latter to facilitate the absorption of cholesterol. The two series of experiments are therefore hardly comparable. It is only fair to say, however, that the daily dose of cholesterol calculated to be equivalent to that contained in the meat diet was so small that one cannot feel certain that it would have produced arterial lesions by itself even if it had been administered in a favorable form. Nevertheless, the experiments as presented do not provide an unassailable demonstration of the ability of protein to aggravate the effects of cholesterol feeding, and they certainly do not prove that the protein in the meat diet was solely responsible for the development of the lesions in the arteries. The direct experiment of feeding a diet rich in proteins and free from cholesterol was not carried out.

In connection with the effect of protein administration, Deicke's experiments [38] are of interest, but they are no more than suggestive. He studied the fluctuations of the blood cholesterol of rabbits following

(*h*) 12, 138.

intravenous injections of colloidal solutions of cholesterol in water. He found the greatest elevations of blood cholesterol following injections of a sol which contained a protein or protein-like substance in addition to cholesterol. He believed that the protein aided in holding the cholesterol in the blood stream. He was unable to produce any vascular lesions by means of parenteral injections, but he used the information gained to explain the results of his feeding experiments in which he had found more pronounced alterations in the arteries following the administration of liver diets than after feeding pure cholesterol in oil.

No conclusions can be drawn from the evidence at hand as to the effect of protein in the diet. The idea that the feeding of protein in conjunction with cholesterol promotes the development of experimental cholesterol arteriosclerosis remains to be substantiated by clearcut experimental evidence. I know of no data to indicate that the administration of sterol-free protein diets can produce arterial lesions similar to those of experimental cholesterol arteriosclerosis.

(*d*) *Blood Pressure.*—The question of the influence of possible fluctuations in the blood pressure of rabbits during cholesterol feeding experiments was first raised by van Leersum [85] and Fahr [47] in 1912. Later it was taken up by Schmidtmann,[i] who reported a moderate rise of the blood pressure in some of her rabbits during the feeding of diets containing liver. She concluded that the cholesterol content of the liver diet was responsible. Deicke [38] and Westphal [180] obtained similar results in rabbits fed with pure cholesterol. However, in none of these series of experiments did the rise in blood pressure occur in all of the rabbits, and it appears doubtful that the fluctuations observed were really outside normal limits.[j] On the other hand, several investigators [k] have reported that observations of the blood pressure at frequent intervals revealed normal values during cholesterol feeding experiments in which severe aortic lesions were produced. Thölldte [154] used Schmidtmann's method for the determination of blood pressure, and although he produced well marked changes in the arteries of his rabbits by feeding them cholesterol in oil, their blood pressures remained within physiologic limits throughout the experiments. He was also able to show that the repeated passage of a stomach tube, which had been suggested as an additional cause of increase in blood pressure, is without perceptible effect. One possible mechanism by which a rise of blood pressure might conceivably be produced is excluded by the work of Okuneff,[114] who showed that the function of the depressor nerves leading from the aortic arch is not

(*i*) 132, 133.

(*j*) Compare 41, 42, 98.

(*k*) 42, 143, 154, 155.

abolished or in any way disturbed by the presence of well developed lesions in the aorta.

It is a safe conclusion that a rise of blood pressure is not necessary for the development of experimental cholesterol arteriosclerosis in rabbits. It is not at all certain that the increases in blood pressure which have been observed are outside the normal range. Furthermore, it has not been demonstrated that a significant rise of the blood pressure can hasten the development of experimental cholesterol arteriosclerosis or increase its severity.

There are recorded in the literature a few experiments purporting to show that increased blood pressure can accelerate the development of experimental cholesterol arteriosclerosis. Starokadomsky and Ssobolew [148] and Anitschkow [5] used the suspension method of Klotz [72] (suspension of the animal by the hindlegs for from 3 to 15 minutes daily) in conjunction with cholesterol feeding. They found fatty deposits in the arteries of their rabbits perhaps a little earlier than when cholesterol was fed without any other treatment. Anitschkow [5] also found that narrowing of the abdominal aorta by means of a ligature slightly accelerated the development of fatty lesions above the constricted point. Moehlig [1] has reported that daily injections of solution of pituitary increase the severity of the vascular lesions produced in rabbits by cholesterol feeding. On the other hand, Bailey [19] found that neither suspension nor injections of solution of pituitary had any effect on the development of arterial lesions in cholesterol-fed guinea-pigs.

It seems unlikely that any of these experimental procedures could have produced a sustained rise of blood pressure. Even the extent of the assumed fluctuations of blood pressure with each repetition of the treatment is a matter for conjecture since no blood pressure determinations were made in any of the experiments. Thus, it is impossible to say exactly what might have been responsible for the rather doubtful acceleration of the rate of development of the lesions in the arteries. In some instances, the upsets in the circulatory mechanism may have been severe enough to amount to actual trauma. In any event, these experiments fall far short of demonstrating clearly that increased blood pressure can hasten the development of experimental cholesterol arteriosclerosis.

(*e*) *Reticulo-Endothelial and Endocrine Systems.*—The production of experimental cholesterol arteriosclerosis in rabbits has been found to be facilitated by splenectomy [m] and by preliminary intravenous injections of india ink [29] which were erroneously supposed to "block" the reticulo-endothelial system. These results might be expected in view

(*l*) 103, 104.
(*m*) 141, 142.

of the fact that in cholesterol-fed rabbits lipoids accumulate in large quantities in the reticulo-endothelial cells.[135] Consequently, a reduction of the functional capacity of the reticulo-endothelial system might well accentuate the effects of cholesterol feeding.

Several investigators[n] have reported that castration promotes the development of experimental cholesterol arteriosclerosis in rabbits. Subtotal suprarenalectomy does not alter their susceptibility to the development of vascular lesions following the feeding of hydrous wool fat.[o] The contradictory results of Moehlig[p] and Bailey[19] regarding the effects of injections of solution of pituitary were mentioned in the discussion of the influence of fluctuations in blood pressure.

The most pronounced effects attributable to endocrine influences are those which follow interference with thyroid function. Extirpation of the thyroid gland has been found to facilitate the development of experimental cholesterol arteriosclerosis in rabbits,[q] while the feeding of dried thyroid gland[r] or of thyroid extract[50] in conjunction with cholesterol retards the progress of the lesions. Thyroxine seems to be less effective in this respect than whole thyroid gland.[158] Thyroidectomy has little effect on the blood cholesterol curve in cholesterol-fed rabbits,[s] but when thyroid preparations are administered with cholesterol to normal rabbits, the resulting hypercholesteremia is much less extreme than that which occurs in control animals fed cholesterol alone.[t] This latter fact suggests that the administration of thyroid preparations in some manner assists the organism in disposing of exogenous cholesterol and in this way exerts its protective action against the effects of cholesterol feeding which would otherwise follow.

A number of recent investigations[u] have demonstrated that the administration of large doses of certain organic iodine compounds or of potassium iodide retards or even prevents the development of experimental cholesterol arteriosclerosis in rabbits. It has been shown further that the administration of potassium iodide greatly diminishes the hypercholesteremia which usually follows cholesterol feeding.[v] The analogy to the effect of feeding thyroid gland immediately suggests the possibility that the iodine compounds act through an influence on thyroid activity. The experiments of Turner and Khayat[159] indicate that this is actually

(*n*) 34, 108, 141, 142.
(*o*) 141, 142.
(*p*) 103, 104.
(*q*) 141, 142.
(*r*) 109, 158.
(*s*) 50, 159.
(*t*) 50, 158.
(*u*) 88, 90, 106, 116a, 139, 158, 159.
(*v*) 139, 158, 159.

the case, for they found that the prophylactic effect of potassium iodide is abolished by thyroidectomy.[v1]

(*f*) *Injury to the Arteries.*—It was mentioned in describing the microscopic features of experimental cholesterol arteriosclerosis in rabbits (section IV *b*) that certain definite alterations occur at the sites of fatty deposits prior to the appearance of lipoids in those areas. The alterations in the intima are inconspicuous, but those in the media can hardly be overlooked and are of such a nature as to be identified as the result of some sort of injury. The fact that these changes precede the appearance of stainable fat is in itself suggestive of a causal relationship. It seems not unlikely that these preliminary changes are responsible for the subsequent accumulation of lipoids in the areas so altered. It is even possible that these preliminary alterations may be indispensable to the process of lipoid accumulation, and evidence in support of this possibility is forthcoming from the experiments to be described in subsequent paragraphs. However, before these experiments are considered, certain time relationships should be called to mind.

In discussing the rôle of cholesterol in the diet, it has been emphasized that a certain minimum period of time must elapse before fatty deposits will make their appearance in the arteries of animals subjected to the administration of cholesterol, even though large doses are employed. In rabbits, lipoid deposits in the arteries have never been produced, so far as I am aware, within less than 20 days from the commencement of experiments which involved no procedure other than the feeding of a cholesterol-rich diet. This lapse of time might conceivably depend on a necessity for building up a sufficient concentration of cholesterol in the blood stream, but the cholesterol content of the blood rises markedly shortly after the commencement of feeding, and before stainable fat appears in the arterial walls, it has usually reached a value of 3 or 4 times the normal, a degree of hypercholesteremia which is known to be much greater than is essential for the development of lipoid deposits in the walls of the arteries. On the other hand, it may be supposed that the delay in the appearance of lipoids in the arterial walls is due to a necessity for the prior development of the alterations in the walls of the vessels mentioned in the preceding paragraph. The latter idea gains support from the fact that experimental injury to the arteries of cholesterol-fed rabbits makes possible the accumulation

(*v*[1]) The recent experiments of Rosenthal (Arch. Path. **18**:827, 1934), combining the administration of cholesterol and that of relatively small doses of iodides in rabbits, yielded results which are quite the opposite of those obtained with massive doses of iodine compounds as outlined. Histologic examinations of the thyroid glands in his animals indicated that the much smaller doses of iodides had decreased thyroid activity. This effect on the thyroid, presumably the reverse of that produced with large doses of iodides, was held responsible for the apparent discrepancy in the experimental results.

of stainable fat in the injured areas in much less than 20 days, or, if the experiment is of longer duration, it permits a much more abundant deposit of lipoids in the damaged regions than in the remaining parts of the arterial tree.

Ssolowjew [145] carried out a series of cholesterol feeding experiments on rabbits in which the carotid artery and aorta were damaged in small areas by cauterization of the adventitial surface. When cauterization was performed at the commencement of the feeding or after it had been in progress for some time, abundant lipoid material was found in the injured parts of the media as a diffuse deposit of fine fatty droplets. This was the case even within 9 days after cauterization, when cholesterol feeding had been carried on for some time previously. The same observation was made at the end of 14 days after a cauterization which was performed coincidentally with the commencement of the cholesterol feeding. In this case no trace of a lipoid deposit was found elsewhere in the arteries.

In a later series of experiments,[146] Ssolowjew found that similar results followed injuries produced in a different way. Rabbits were prepared by an operation in which the carotid artery was dissected free and brought out to a position just beneath the skin. It was found that this procedure with or without subsequent massage of the artery produced tiny transverse tears in the inner part of the wall of the vessel. When animals prepared in this way were fed with cholesterol in oil, the injured areas in the carotid artery, if not completely healed, became impregnated with lipoids, while the adjacent normal parts of the vessel remained free from stainable fat. The fatty material was deposited in relation to the intercellular ground substance in the damaged parts of the media, and fat was also contained in scattered phagocytic cells. In one other experiment in which cholesterol feeding had been started some time before operation, the manipulated carotid artery showed lipoid accumulations in the injured areas at the end of only 4 days from the time of operation, although the opposite carotid artery which had been left undisturbed showed no lipoid deposits.

Perhaps at this point a less artificial type of local injury may be brought into consideration for its localizing and accelerating effect on the accumulation of lipoids in the walls of arteries. Such injured areas are conveniently furnished by the occurrence of the common medial variety of spontaneous lesion in the aortas of rabbits. As has been mentioned, these areas of necrosis and calcification in the media are always characterized by an almost complete absence of stainable fat. However, when cholesterol feeding is instituted, the injured areas, excepting their calcified parts, rapidly become impregnated with an abundant deposit of fine fatty droplets or granules. Thus, in one of my own experiments,[45] a spontaneous medial lesion in the aorta of a rabbit showed a

diffuse deposit of fine anisotropic lipoid granules at the end of 14 days of cholesterol feeding. Another animal showed a most abundant deposit of anisotropic fatty material in a similar medial lesion after only 25 days of feeding. In neither of these animals was there any trace of lipoid deposit in the remaining parts of the arteries. With a diet similar to that used in these rabbits, the earliest fatty deposit appeared in an otherwise normal aorta only after 38 days. After protracted feeding of cholesterol in rabbits, an area of spontaneous medial necrosis is marked out even in the gross by a fat-laden circular thickening of the intima, which microscopically shows the features characteristic of the intimal lesions of experimental cholesterol arteriosclerosis. The injured media beneath shows dense lipoid infiltration.

All of these experiments demonstrate clearly that the damaged tissue, especially the altered intercellular ground substance, in injured areas in the walls of arteries has a marked predilection for the accumulation of lipoids. The injured tissues become infiltrated with lipoids in a much shorter time than is necessary for the development of a similar infiltration in a previously undamaged vessel. Furthermore, it is clear that hypercholesteremia (together with any other necessary changes in the blood) of sufficient degree to permit the deposition of lipoids in the arterial walls can exist without the development of any lipoid deposits in normal parts of the arteries. Under such conditions at least, injury to the wall of the vessel proves to be an indispensable factor without which lipoid deposits cannot or do not occur.

The arteries of rabbits can be injured also by injections of a variety of substances, and this means may be employed for the purpose of studying the effects of injury on the development of experimental cholesterol arteriosclerosis. Intravenous injections of epinephrine and nicotine have been used in this way.[w] Either epinephrine or nicotine alone can produce severe lesions in the rabbit's aorta simulating very closely those which follow the administration of barium chloride, hydrastine, digitalin, diphtheria toxin, etc.[x] The exact way in which the injury is produced is still uncertain, but it seems probable that these substances act more or less directly on the arteries rather than through an effect on the blood pressure.[y] The most conspicuous features of the lesions in the aorta are necrosis and subsequent calcification of the media. Changes in the overlying intima are relatively slight but definite, consisting of a swelling of the subendothelial ground substance and sometimes fraying of the internal elastic lamina. Intimal proliferation may occur in more advanced lesions, but throughout the process there is an almost complete absence of stainable fat in both the intima and the media.

(*w*) 5, 37.
(*x*) 71, 127.
(*y*) 37, 71, 83, 127.

When injury of this sort is combined with cholesterol feeding in rabbits, the accumulation of anisotropic lipoids in the walls of the aorta is considerably accelerated, as has been shown by Anitschkow [5] and Danisch.[37] In their experiments, in which epinephrine and nicotine were the damaging agents employed, lipoid deposits were constantly associated with the lesions caused by the epinephrine or the nicotine. The lipoid deposits were found in the necrotic parts of the media and in the swollen subendothelial layer of the intima over the medial lesions.

The administration of large doses of irradiated ergosterol (viosterol, vigantol) also is capable of injuring the aortas of rabbits, producing lesions almost identical with those which follow repeated injections of epinephrine. This fact was first demonstrated by Kreitmair and Moll [79] in 1928, and their observation has since been confirmed by many others.

Schmidtmann [134] found that the administration of vigantol in conjunction with cholesterol feeding promoted the development of experimental cholesterol arteriosclerosis in rabbits. Her observations were confirmed by Pfleiderer [121] in a more extensive study. In his experiments, the most abundant deposits of lipoids in the walls of the arteries were always found in association with the easily recognizable vigantol lesions, the severity of which seemed to govern the abundance of the lipoid accumulations. Pfleiderer concluded that the arterial injury produced by the vigantol had caused a greatly increased predilection for the accumulation of lipoids in the arterial walls.

Harrison's experiments [59] with the combined administration of viosterol and cholesterol were arranged somewhat differently, and the results demanded a somewhat different interpretation. His rabbits were given a preliminary course of viosterol and calcium lactate by mouth. This treatment produced densely calcified localized lesions in the media of the aorta. The administration of viosterol was discontinued, and after an interval of ten days a prolonged course of cholesterol feeding was instituted. At the termination of the experiments, the old calcified viosterol lesions showed no lipoid deposits except around their margins, in the parts of the aortic wall immediately adjacent, where the typical fatty lesions attributable to cholesterol feeding were constantly located. The explanation offered for this peculiar localization seems plausible. Harrison argued that the vessel wall at the margins of the rigid calcified plaques must have been subjected continually during life to mechanical distortion occasioned by the systolic distention and diastolic recoil of the aorta. He concluded that these excessive movements within the vessel wall were responsible for the localization of the cholesterol lesions in these areas. It may be thought that this explanation necessitates the recognition of a mechanical factor as a predisposing and localizing influence quite distinct from other forms of injury to the walls of arteries. However, Harrison's conclusion really implies only this, that sufficiently violent mechanical distortion of the arterial wall can produce

a local injury which is adequate to determine the localization of experimental cholesterol arteriosclerosis.

There is some evidence to indicate that bacterial infections may also have an influence in promoting the development of experimental cholesterol arteriosclerosis in rabbits. Saltykow [129] carried out a large number of experiments on rabbits which were fed on milk and bread and which, in addition, were subjected to intravenous injections of old cultures of staphylococci. The cholesterol content of milk is not high, but the experiments extended over periods up to 2 years, so that the animals probably received a large quantity of cholesterol in that time.[128] Saltykow believed that the typical fatty arterial lesions which he produced were caused primarily by the cholesterol contained in the milk, but he concluded that the injections of staphylococci had a definite effect in accelerating their development and increasing their severity. This conclusion is supported by the recent results of Benson, Smith and Semenov.[24] These investigators fed a group of rabbits with cholesterol for 9 months, the total quantity fed averaging 65 Gm. for each animal. No arterial lesions were found in this group, evidently because the cholesterol was administered as a dry powder mixed with oatmeal and was therefore probably not absorbed in sufficient quantity to produce lesions by itself. However, the same daily dose of cholesterol given in the same way to another group of rabbits from the same litters as the first produced typical fatty lesions of the intima in a large proportion of the rabbits within 7 months when the cholesterol feeding was accompanied by intravenous injections of streptococci. The animals received a total quantity of cholesterol averaging only 46 Gm. for each. Injections of streptococci alone did not produce similar lesions, but frequently did result in visible injury to the arteries in the form of an acute arteritis.

The experimental results which have been quoted in the preceding pages are in agreement in showing that the development of experimental cholesterol arteriosclerosis in rabbits is facilitated by the introduction of various noxious agents which have in common the ability to injure the arterial walls, as demonstrated in control experiments.

It seems evident that the alterations in the walls of the vessels attendant on the injury were responsible for the more rapid development of the lipoid deposits, since the latter were found constantly in association with whatever visible lesions were present in the arterial walls. For example, in the experiments with epinephrine and nicotine, the fatty deposits in the intima and media occurred especially in those parts of the aorta which showed the characteristic epinephrine or nicotine lesions. However, the changes in the intima in such areas up to the time of the earliest appearance of lipoid deposits there, were not more marked than those which have been described as occurring in the early stages of the lesions which result from cholesterol feeding alone, namely, a swelling of the

subendothelial ground substance and sometimes fraying of the internal elastic lamina. Thus, the fact that histologic changes in the intima prior to the advent of lipoid deposits are not striking and conspicuous does not preclude the possibility that they represent the result of definite injury whether a known damaging agent has been administered or not.

The results of all recorded experiments which bear on the question of the influence of injury to the arteries are in agreement. They indicate that injury, however produced, will facilitate the development of lipoid deposits in the arteries of rabbits fed cholesterol-rich diets. Injury to the arteries in conjunction with cholesterol feeding is able to bring about a much earlier initiation of the process of lipoid deposition than ever occurs following cholesterol feeding alone. It has been shown that the general conditions in the blood may be suitable for the development of lipoid deposits without the occurrence of any such deposits in normal parts of the arteries. Lipoid deposits occur especially in relation to the altered intercellular ground substance in injured portions of the walls of the vessels, and they develop much more rapidly in such areas than in previously undamaged parts of the arteries. However, in the intima of the rabbit's aorta, the changes found at the time of the earliest appearance of lipoid deposits are the same in kind and degree whether cholesterol has been administered with or without an additional agent which is known to be injurious to the rabbit's arteries and which accelerates the deposition of lipoids in the arterial walls.

Correlation of all of these facts can be accomplished only by assuming that lipoid deposits in the arteries are always preceded by preliminary local alterations in the walls of the vessels which are indispensable to the formation of the lipoid accumulations, and which therefore constitute the initial stage of the development of experimental cholesterol arteriosclerosis. Moreover, it seems highly probable that these primary local changes in the arterial walls represent the result of some sort of injury. These conclusions are strongly suggested by the evidence at hand, and they are confirmed by other considerations to be discussed in a subsequent part of this paper (section VIII). I have already pointed out the probability that hypercholesteremia of itself is not solely responsible for the development of experimental cholesterol arteriosclerosis, and that some other factor is also necessary. On the basis of the evidence adduced here, it seems highly probable that some form of injury to the arterial walls is the second essential factor which operates in conjunction with hypercholesteremia in the production of experimental cholesterol arteriosclerosis in rabbits.

VII. SUMMARY OF IMPORTANT EXPERIMENTAL DATA

Lipoid accumulations in various organs and fatty lesions of the arteries can be produced in rabbits by feeding diets which have a con-

siderable content of cholesterol. The general distribution of the arterial lesions has been indicated, and the aortic lesions have been described in detail in section IV of this paper. The individual lesions are discrete and have a yellowish color due to the lipoids which accumulate in them. The lipoid deposits are usually to be found first in the intima in the form of minute fatty droplets lying in the subendothelial ground substance, which has previously assumed a greatly swollen appearance. The earliest lipoid accumulations sometimes occur in the inner layers of the media where focal areas of muscle necrosis appear, and subsequently become impregnated with lipoids. However, the medial lesions are usually associated with corresponding changes in the overlying intima. Much of the fatty material, whether in the intima or in the media, is anisotropic. Soon after the advent of lipoids in the intima, large fat-containing phagocytic cells ("foam cells") and fibroblasts appear in varying numbers, producing an increasing thickening of the intima. The lesions in the media may become extensive through progressive destruction of muscle and elastic tissue followed by lipoid impregnation of the necrotic areas. If cholesterol is withdrawn from the diet, well developed lesions in the intima take on a more fibrous character, and the lipoids gradually diminish in abundance. These lesions of "experimental cholesterol arteriosclerosis" are not dependent on spontaneous disease of the arteries for their development.

The feeding of cholesterol-rich diets probably can produce similar lesions in the arteries of guinea-pigs, and the development of these lesions, too, is thought to be independent of spontaneous arterial disease. The lesions found in the arteries of pigeons, chickens, parrots, mice, rats and goats following cholesterol feeding do not occur with any constancy, and they cannot be freed from a strong suspicion of spontaneity. They are therefore excluded from further consideration. All attempts to produce lesions in the arteries of cats, dogs, foxes and monkeys by feeding cholesterol-rich diets have been entirely without success.

In rabbits, experimental cholesterol arteriosclerosis can be produced by feeding solid cholesterol; it has never been produced with the feeding of any other lipoid or with sterol-free diets. It is concluded, therefore, that cholesterol feeding is an essential factor in the production of the arterial lesions. However, the concomitant feeding of neutral fats and other lipoids greatly accelerates the development of experimental cholesterol arteriosclerosis and hence is an important factor. The factor of time is also of importance since even microscopic fatty deposits in the arteries have never been produced in less than 20 days by feeding cholesterol-rich diets, and usually a considerably longer time is required.

During the course of most experiments with cholesterol feeding in which lesions in the arteries are produced, marked hypercholesteremia

occurs, but the severity of the arterial lesions does not run strictly parallel with the elevation of the level of the blood cholesterol. Indeed, well marked hypercholesteremia can develop following cholesterol feeding without producing any lesions in the arteries. On the other hand, experimental cholesterol arteriosclerosis may appear in the presence of a relatively slight increase in the cholesterol content of the blood. However, it has not been demonstrated that the characteristic arterial lesions can be produced without any elevation of the blood cholesterol above its normal level. It is assumed, therefore, that some degree of hypercholesteremia is an essential factor in the development of experimental cholesterol arteriosclerosis in rabbits. The hypercholesteremia which follows cholesterol feeding is accompanied by a corresponding elevation of other lipoids in the blood, a rough index of which is given by the increase in its cholesterol content. The total result is hyperlipemia, i. e., an increase of all the blood lipoids above their normal limits.

At present there is no satisfactory evidence to substantiate the idea that the feeding of protein in conjunction with cholesterol facilitates the development of experimental cholesterol arteriosclerosis.

Experimental cholesterol arteriosclerosis can develop in rabbits without any rise of the blood pressure beyond the normal limit. Furthermore, there is no good evidence to show that an increase of the blood pressure can affect the progress of the lesions in the arteries.

Splenectomy, castration and thyroidectomy are said to accelerate the development of experimental cholesterol arteriosclerosis in rabbits. The administration of dried thyroid gland or of other thyroid preparations retards the progress of the arterial lesions. The administration of potassium iodide and of certain organic iodine compounds has a similar effect, but this is abolished by thyroidectomy.

If the feeding of cholesterol-rich diets is combined with some other procedure which is known to injure the arteries, the development of experimental cholesterol arteriosclerosis is greatly accelerated. This is true of all forms of injury which have been employed. Deposits of anisotropic lipoids occur especially in relation to the altered intercellular ground substance in the injured portions of the wall of the vessel, and they develop much more rapidly in these areas than in previously undamaged parts of the artery. Such injured areas can become impregnated with lipoids during a period in which no lipoid deposition has occurred in the remaining normal parts of the wall of the vessel. It seems highly probable that some form of injury to the arterial walls is the second essential factor which operates in conjunction with hypercholesteremia in the production of experimental cholesterol arteriosclerosis in rabbits.

[The bibliography will be found at end of the last instalment, in the August issue.]

(To be concluded)

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, etc.—Marcos Fernan-Nunez, professor of pathology and bacteriology in Marquette University, Milwaukee, has been elected a member of the National Academy of Medicine of Spain.

According to *Science,* Simon Flexner, director of the laboratories of the Rockefeller Institute for Medical Research since the opening of the institute in 1903, has presented his resignation, to take effect on the appointment of his successor.

On the retirement of Milton J. Rosenau from active teaching as professor of preventive medicine and hygiene in the Harvard University Medical School, his portrait by Jacob Binder has been presented to the school by his colleagues.

The Lister Medal for 1936, which is awarded in recognition of distinguished contribution to surgical science, has been granted to Sir Robert Muir, professor of pathology in the University of Glasgow.

Perry J. Melnick, formerly chief resident pathologist in the Cook County Hospital, Chicago, has accepted the position of pathologist in the Decatur and Macon County Hospital, Decatur, Ill.

Howard W. Florey, professor of pathology of the University of Sheffield, has been appointed professor of pathology at the University of Oxford.

At the London Hospital William Bulloch has resigned from the Goldsmiths' Company's chair of bacteriology and has been succeeded by S. P. Bedson.

Nine fellowships in medicine, including two renewals, for study in the United States and abroad during the year 1935-1936, were awarded at the spring meeting of the Medical Fellowship Board of the National Research Council in Washington, D. C. The successful candidates are: Oscar E. Block Jr., Berry Campbell, Jack M. Curtis (renewal), Windsor C. Cutting, Samuel Gurin, Robert E. Johnson, Benjamin F. Miller (renewal), E. Byron Riegel, Morris F. Shaffer.

Society News.—The fifth annual convention of the Biological Photographic Association will be held at the Stevens Hotel, Chicago, on September 12, 13 and 14. Further details and copies of the program may be had by writing Ralph P. Greer, chairman of the program committee, Box 266, Hines, Ill.

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

THE CORRELATION OF MINERAL METABOLISM AND THE VEGETATIVE NERVOUS SYSTEM IN THYROID DISEASE. JACOB KLEIN, Ann. Int. Med. **8**:798, 1935.

On the assumption that in thyrotoxic conditions there is a negative calcium balance that may explain the alterations in tone of the vegetative nervous system Klein studied the thyroids of fifty rats under varying dosages of iodine, calcium and epinephrine. Noting the parallelism between the iodine content and the size of the follicles, he took the average measurement of 100 follicles as the "follicular index." Normal rats had a follicular index of 251 microns; those with a diet deficient in iodine, 48 microns; those given a diet deficient in iodine plus a daily stimulation with epinephrine, 187 microns; those receiving 3 per cent calcium in addition to the iodine-deficient diet and epinephrine, 211 microns. It is concluded (1) that a dietary deficiency of iodine causes atrophy of the thyroid follicles and loss of colloid, (2) that stimulation of the sympathetic nerves with epinephrine in the presence of a deficiency of iodine causes hyperactivity of the thyroid epithelium, and (3) that administration of calcium promotes storage of colloid and neutralizes the harmful effects of the stimulation of the sympathetic nerves.

FRANK R. MENNE.

THE EFFECT OF SODIUM SALTS IN SUSTAINING THE SUPRARENALECTOMIZED DOG. G. A. HARROP ET AL., J. Exper. Med. **61**:839, 1935.

A group of experiments is reported in which bilaterally adrenalectomized adult male dogs were maintained in apparently normal condition over prolonged periods, up to five months, without use of any adrenal gland preparation or extract by administration of sodium chloride and sodium bicarbonate alone. Withdrawal of the salts produced typical adrenal insufficiency. Further evidence is presented in these experiments in support of the view that the adrenal cortical hormone in the adult male dog is concerned with the regulation of sodium excretion by the kidney and thus eventually with the proper maintenance of water balance in the organism. It has no direct influence on carbohydrate metabolism. The reciprocal changes in the plasma concentrations of urea and potassium which take place as the concentrations of plasma sodium and chlorides vary are pointed out as furnishing a mechanism whereby abrupt alterations in osmotic pressure are dampened and the volumes of fluids in extracellular and intracellular compartments more efficiently stabilized.

FROM THE AUTHORS' SUMMARY.

HISTOLOGIC CHANGES IN THE KIDNEYS OF ADRENALECTOMIZED RATS. S. L. SIMPSON and V. KORENCHEVSKY, J. Path. & Bact. **40**:483, 1935.

In adrenalectomized rats degeneration of the second convoluted tubules with some degree of consequent regeneration was found. Daily subcutaneous administration of cortical extract diminished or prevented these changes. Of two recovering rats one presented normal kidneys and the other moderately severe changes, suggesting that apparent recovery may be associated with latent adrenal insufficiency.

FROM THE AUTHORS' SUMMARY.

THE THYMUS IN CHEMICAL INTOXICATIONS. G. WORMS and H. P. KLOTZ, Ann. d'anat. path. **11**:1, 1934.

The changes in the thymus gland following injections of phosphorus, mercuric bichloride, methyl alcohol and arsenic were studied in dogs, rabbits and guinea-

pigs. The most extensive studies were made with phosphorus, but in general the action of all the agents was fundamentally the same. Atrophy of the thymus takes place by a series of stages. If weakly toxic doses are injected over a long period, these stages can be observed in succession. First there is an infiltration of the normally clear medullary zone by lymphocytes escaping from the intralobular artery. At the same time the cortical substance becomes cleared because of the destruction of a large number of the epithelial thymic cells, resulting in an inversion of the lobule. If the toxic agent is now withdrawn the thymus gradually returns to its original state. In prolonged administration of the poison, however, the thymic lobules become segmented into smaller and smaller subdivisions by trabeculae of a colloid substance derived from the necrotic thymic cells. There is no fibrosis or fatty change. Hassall's corpuscles usually disappear also. The changes resemble those produced by the x-rays and other agents.

PERRY J. MELNICK.

RENAL LESIONS PRODUCED BY GOLD SALTS. A. KÁLLÓ, Ann. d'anat. path. **11**:21, 1934.

Eight different types of gold salts were administered to sixty guinea-pigs, rabbits and rats. Normal as well as tuberculous animals were used. The dosages included therapeutic, toxic and fatal doses. The kidneys were examined microscopically. The effects of toxic and fatal doses consisted, on the one hand, of intense hyperemia, diapedetic hemorrhages and cellular infiltration and, on the other hand, of marked degenerative changes and even necrosis of the tubular epithelium, especially of the convoluted tubules. These findings were always associated with albuminuria or suppression of the urine. Therapeutic doses produced mainly circulatory changes, but also slight parenchymatous changes. The author therefore advises caution in the therapeutic use of gold salts.

PERRY J. MELNICK.

DEFICIENCY OSTEITIS IN CHICKENS KEPT IN CAGES AND ITS RESEMBLANCE TO OSTEITIS FIBROSA WITH HYPERTROPHY OF THE PARATHYROIDS. C. OBERLING and M. GUÉRIN, Ann. d'anat. path. **11**:97, 1934.

In eighty chickens kept in cages for varying periods up to a year marked deformity of the skeleton was observed, of a type familiar to chicken raisers and variously designated as rickets, osteoporosis or osteomalacia. Etiologically, sunlight and vitamin D were not necessarily of importance. The factor involved was an absence of gravel in the ration, apparently a mineral deficiency. The bones were deformed, swollen, hyperemic and soft so that they were easily cut, and often had many cysts. Microscopically, two distinct types of lesions were found. One type resembled rickets and osteomalacia, with gradual atrophy of the bony trabeculae under the influence of the dilated and encroaching vascular spaces. Sometimes only atrophy was seen; in other cases osteoid tissue was laid down in an attempt at regeneration. Most of the chickens in which this type developed had been kept in darkness. The second type of lesion resembled osteitis fibrosa. The narrow bony trabeculae presented many Howship's lacunae associated with many osteoclastic giant cells and a cellular inflammatory tissue. Cysts were frequent in this type. Occasionally both types of lesions were found in the same fowl. In most cases of both types there was an associated hypertrophy of the parathyroid glands. Microscopically, these glands were either typical, atypical or adenomatous; the structure could not be correlated with the type of lesion.

The authors believe that the different types should not be considered as separate entities. Separate etiologic factors have not been established for them. They are different reaction forms of bone tissue depending on various factors. The parathyroid hypertrophy is considered as secondary to the disturbance of the mineral metabolism and reversible. In osteitis fibrosa the stimulus to hypertrophy results

in an adenomatous proliferation which is irreversible. Thus, rickets, osteomalacia and osteitis fibrosa are related in that each fundamentally is a disturbance of mineral metabolism.

PERRY J. MELNICK.

NEW FORMATION OF CAPILLARIES IN INFLAMMATORY TISSUE. J. GOLDNER, Ann. d'anat. path. **11**:461, 1934.

Inflammatory tissue produced aseptically by injecting foreign material intraperitoneally into guinea-pigs was fixed in Zenker's fluid, sectioned and stained with silver stains as well as by hematologic methods. Goldner considers three factors to be important in the new formation of capillaries in such tissue, namely, reticulin fibers, histiocytes and endothelium-formative cells. The reticulin fibers are of primary importance. They are usually laid down by histiocytes and determine the location and direction of the new-formed capillary; that is, they form a kind of framework for it. The histiocytes remain as an incomplete perithelial sheath or adventitia (cells of Marchand). The endothelium-formative cells, which may be either fibroblasts or monocytes, then form an endothelium for the capillary. Finally, secondary anastomosis of the new capillaries with the blood vessels in the periphery occurs. This evidence seems to contradict the classic theory that new capillaries bud off from preexisting ones.

PERRY J. MELNICK.

STATES OF INHIBITION OF THE BONE MARROW. J. E. W. BROCHER, Ann. d'anat. path. **11**:585, 1934.

Brocher points out that agranulocytosis is not a specific disease (so-called agranulocytic angina of Schultz), but is a state of inhibition of the bone marrow produced by various acute infections and by poisons. He reports several cases in which typical agranulocytosis (sometimes associated with hemorrhage) developed in patients following treatment with gold salts, bismuth or arsenicals or after prolonged exposure to benzene and other volatile organic poisons, x-rays and radio-active substances. The condition depends on failure of the normal reactivity of the bone marrow.

PERRY J. MELNICK.

EFFECT OF HYPOPHYSECTOMY ON THE PHALANGES OF THE SALAMANDER. K. SCHAEFER, Virchows Arch. f. path. Anat. **293**:562, 1934.

In larval salamanders at the time of metamorphosis the toes become actually shortened. This is due to chondrolytic atrophy of the cartilaginous mass at the distal end of the terminal phalanges. Hypophysectomy of the larvae leads to abnormal enchondral ossification followed by lacunar resorption; in this process many giant cells are formed. Regeneration of amputated extremities or toes is delayed in hypophysectomized animals.

O. T. SCHULTZ.

MORPHOLOGIC EFFECTS OF SPLENIC AUTOLYSATES. G. DERMAN and S. LEITES, Virchows Arch. f. path. Anat. **293**:599, 1934.

Repeated injection of the thermostabile products of autolysis of splenic tissue into rabbits and guinea-pigs led to activation of the reticulo-endothelial system of the spleen and liver. This action was increased if the splenic tissue was irradiated before autolysis. Autolysates of irradiated and nonirradiated splenic and muscle tissue caused regenerative changes in the bone marrow with increase in the number of megakaryocytes and slight degenerative changes in the parenchyma of the liver and kidney; these changes are considered nonspecific. The autolysate of the spleen of a patient with myeloid leukemia led, in the rabbit, to marked proliferation of the reticulo-endothelial system of the liver and spleen and to areas of myeloid metaplasia in the liver. The autolysate of the spleen of a patient with Banti's disease caused fibrosis of the liver in the rabbit.

O. T. SCHULTZ.

Pathologic Anatomy

HISTOLOGY OF THE MIDOLIVARY REGION OF THE MEDULLA OBLONGATA IN THE NEW-BORN INFANT. MYRTELLE M. CANAVAN and FREDERICK A. HEMSATH, Am. J. Dis. Child. **49**:101, 1935.

The vessels seem inadequate in number to nourish so important a structure as the medulla oblongata. In the new-born infant ependymal cells are of greater length than in older persons, averaging 20 microns. Subependymal glia cells are more abundant in the new-born than in the aged. Oligodendroglia cells are slightly more in evidence midway between the olivary bodies, where myelin is laid down at an early stage. In ten premature infants these cells represented 55 per cent of the cells; in ten babies born at term, 48 per cent. Choroid plexuses may have various foci of heaping up of epithelial cells in their tufts. Many cells are present at the surface of the folia of the cerebellum in the new-born.

AUTHORS' SUMMARY.

ENLARGEMENT OF THE PARATHYROIDS IN RENAL DISEASE. A. M. PAPPENHEIMER and S. L. WILENS, Am. J. Path. **11**:73, 1935.

The mean weights of the parathyroid glands in a series of patients over the age of 10 years with miscellaneous non-nephritic diseases were 27 mg. for the upper and from 31 to 32 mg. for the lower gland. The mean combined weight was 118 mg. In the male glands there was no change correlated with advancing age. In the female gland there was an increase in weight of approximately 22 per cent during the active sexual period; after 45 years there was a decline of weight to figures corresponding with those of the series as a whole. The enlargement was not correlated with pregnancy. The mean weight of the parathyroids in various types of chronic renal disease exceeds that in non-nephritic diseases. In an unselected series of cases this increase in mean weight is approximately 50 per cent; in cases of advanced renal lesions the increase amounts to more than 100 per cent. The increase in the weight of the parathyroids is roughly proportional to the severity and extent of the renal lesions and to the intensity of the clinical signs of renal insufficiency. Usually three or four of the glands share in the enlargement.

FROM THE AUTHORS' SUMMARY.

ATYPICAL AMYLOID DISEASE. D. PERLA and H. GROSS, Am. J. Path. **11**:93, 1935.

Three unusual cases of amyloid disease are reported in which an etiologic factor was not demonstrated. The first was that of a woman, 53 years of age, with extensive amyloid disease of the heart, tongue, gastro-intestinal tract and other organs, who died of congestive heart failure. The second was that of a girl, 16 years of age, with extensive amyloid deposits in the kidneys, liver and adrenals, who died of uremia. She had ankylosis of one joint without any evidence of suppuration. The third case was that of a woman, 63 years of age, with amyloid contracted kidneys, who eventually died of uremia. She had severe coronary sclerosis with old occlusion of the descending branch of the left coronary artery and healed infarction of the left ventricle. The amyloid disease was limited to the kidneys. In view of the absence of any apparent suppuration it is suggested that this peculiar disturbance in protein metabolism may be independent of destruction of tissue.

FROM THE AUTHORS' SUMMARY.

HISTOGENESIS OF SUBACUTE LYMPHATIC LEUKEMIA. J. STASNEY and H. DOWNEY, Am. J. Path. **11**:113, 1935.

With three biopsies taken at different times, the histogenesis of a case of subacute lymphatic leukemia was followed. In the early stages, a lymph node from the inguinal region showed diffuse hyperplasia of the syncytial reticulum cells. Many of these cells showed beginning basophilia in the cytoplasm. At the same

time the peripheral blood contained a number of larger cells with cytoplasm of a type that is characteristic of the reticulo-endothelial cell, but with a nucleus of lymphocytic pattern, indicating the heteroplastic origin of lymphocytes from the reticulum. Later an inguinal lymph node presented a dense mass of immature lymphocytes in the medullary region, while the cortical germ centers were still preserved. The immature lymphocytes, therefore, originate in the medulla rather than in the germinal center of the follicles, and the diffusely distributed syncytial reticulum is the mother tissue for these immature lymphocytes.

FROM THE AUTHORS' SUMMARY.

HEPATO-ADRENAL NECROSIS WITH INTRANUCLEAR INCLUSION BODIES. G. M. HASS, Am. J. Path. **11**:127, 1935.

The case of a 7 months premature infant afflicted with a fatal disease characterized by hepato-adrenal necrosis and intranuclear inclusion bodies in the parenchymatous cells of the liver and adrenal cortex is described. It is assumed that the unique lesions must have been produced by a filtrable virus. No record of a similar case has been found in the literature.

FROM THE AUTHOR'S SUMMARY.

MYOCARDIAL LESIONS IN SUBACUTE BACTERIAL ENDOCARDITIS. O. SAPHIR, Am. J. Path. **11**:143, 1935.

Myocardial changes were encountered in thirty-five cases of subacute bacterial endocarditis. These changes may be summarized as cloudy swelling, fatty degeneration, petechial hemorrhages, acute myocarditis, foci of necrosis and abscesses, areas of perivascular acute and chronic (nonspecific) inflammation, minute infarcts, emboli in branches of the coronary arteries, Aschoff bodies and perivascular fibrosis. Minute infarcts were the most commonly encountered and the most characteristic lesions. Bracht-Wächter bodies are discussed and the conclusion reached that this term signifying specific lesions should be discarded because of the uncertainty of just what constitutes a Bracht-Wächter body.

FROM THE AUTHOR'S SUMMARY.

ANNULAR PANCREAS. J. B. MCNAUGHT and A. J. COX, Am. J. Path. **11**:179, 1935.

Another case of annular pancreas is recorded, bringing the total number of reported cases to forty-four. Annular pancreas is undoubtedly a developmental anomaly of the ventral pancreatic anlage. Our case was that of a man, 70 years of age, with several congenital anomalies but with no complaints referable to them. A rapid and simple method of accurately tracing the duct system of the pancreas by means of an injection of a bismuth suspension is described.

FROM THE AUTHORS' SUMMARY.

FRAGILITY AND MATURATION OF RETICULOCYTES. CAMILLE MERMOD and W. DOCK, Arch. Int. Med. **55**:52, 1935.

The reticulocytes, particularly the young (heavily stained) ones, are more easily destroyed in shed blood and in the circulating whole blood by saponin (and by citrate and oxalate in whole shed blood) than are the other red cells. The maturation of reticulocytes in the circulating blood is not proved; it seems not to occur on any significant scale. The occurrence of a reticulocytic crisis early in the treatment of anemia is probably an unavoidable evil associated with a change in the structure and function of the bone marrow. This precedes and delays the rise in the red cell count.

AUTHORS' SUMMARY.

PARAPLEGIA OF PREGNANCY (SUBACUTE COMBINED DEGENERATION OF THE CORD). GEORGE B. HASSIN and ABRAHAM ETTLESON, Arch. Neurol. & Psychiat. **32**:1273, 1934.

In a girl, aged 19, who gave a history of gonorrhea and syphilis and who had been pregnant five months spastic paraplegia developed without marked paresthesia or definite sensory disturbance. No signs of pernicious anemia or of cord compression were present. The condition grew worse and after a spontaneous premature delivery of the child at the eighth month the patient died with signs of septicopyemia. Necropsy revealed far advanced degeneration of the lateral columns, foci of septicopyemia in the brain and the mild degeneration of the posterior columns and changes in the anterior horn cells typical of so-called axon reaction (swollen cell bodies, misplacement of the nucleus to the periphery, retraction of the dendrites and chromatolysis).

AUTHORS' ABSTRACT.

THE CENTRAL NERVOUS SYSTEM IN MUSHROOM POISONING. ELI MARCOVITZ and BERNARD J. ALPERS, Arch. Neurol. & Psychiat. **33**:53, 1935.

The authors describe central nerve changes in a woman aged 26 and a man aged 27 who died from mushroom poisoning (Amanita phalloides) on the fifth and seventh days, respectively. The changes in the brain were punctate hemorrhages and swollen, fragmented and vacuolated ganglion cells, some with pyknotic nuclei and containing lipoids, which were present also in the neuroglia, microglia, vascular endothelium and ependymal cells. In addition, there were scattered inflammatory changes in the form of marked perivascular infiltrations. The authors classify the changes as toxic encephalopathy combined with inflammatory phenomena which, in their opinion, are not secondary but primary, in no way different histologically from an infectious nonsuppurative encephalitis. The temperature in one case rose as high as 106 F.; in the other case it was not given.

GEORGE B. HASSIN.

CEREBRAL CHANGES IN GASTRO-INTESTINAL INFECTIONS WITH TERMINAL CACHEXIA. LEO ALEXANDER and T. T. WU, Arch. Neurol. & Psychiat. **33**:72, 1935.

Alexander and Wu studied the changes in the brains of twenty patients in whom cachexia developed as the result of various types of dysentery and intestinal tuberculosis (five cases). The ages of the patients varied from 6 months to 49 years. One patient was a premature baby. The durations of the diseases ranged from a few days to several months. Some patients had had recurrent attacks of diarrhea for as long as ten years. The clinical manifestations were in many cases psychotic—delirium, maniacal outbursts and Korsakoff's syndrome; convulsions were a prominent feature in a few cases, aside from severe somatic disturbances. The cerebral changes were mainly regressive—parenchymatous and circulatory. Hemorrhages and inflammatory phenomena were entirely absent. Ischemic foci in the third and fifth cortical layers were the most outstanding changes. In such foci ganglion cells were few; many were replaced by small glial cells; some cells disappeared without leaving traces; other cells were as shadows or were "pyknotic" or "pyknomorphic," but foci of complete ischemic necrosis were not present. The other changes were what the authors call "soaking," as they resembled the changes that can be produced in the ganglion cells by soaking fresh brain tissue in distilled water. Such changes show as marked argentophilia of the nuclei and crumbling of the neurofibrils. In five cases so-called pseudo-atrophy of the brain was observed, especially of the frontal lobe, without fibrosis of the meninges, which in the authors' opinion was due to emaciation.

GEORGE B. HASSIN.

MENINGO-ENCEPHALITIS CAUSED BY CYSTICERCUS CELLULOSAE. VERA B. DOLGOPOL and MARCUS NEUSTAEDTER, Arch. Neurol. & Psychiat. **33**:132, 1935.

A Mexican woman, aged 30, had been suffering for a year from headaches which were complicated within five months by visual disturbances, vomiting and convulsive seizures of the left arm and leg. Present were Argyll Robertson pupil, papilledema of the left fundus, edema of the eyelids, and pleocytosis and increase of globulin in the spinal fluid, which contained 24 per cent eosinophils (the blood showed 10 per cent of eosinophils). Jacksonian attacks developed in the right half of the body preceded by hyperesthesias of the right upper and lower extremities. Because of gradually increasing visual disturbances, an opening was made in the right temporal region and a small mass was removed from under the dura. Five weeks later necropsy revealed chains of vesicles attached to the meninges of the sylvian fissure and both sides of the pons; the majority of the cysts were empty or contained a hyaline substance but no parasites. The capsules of the cysts showed marked reactive inflammatory phenomena, as did the meninges and the cerebral parenchyma. The vessels of the brain were not only infiltrated with lymphocytes and plasma cells but showed a marked endarteritis which almost obliterated the lumen of some arteries. This occlusion was evidently the cause of the softening of the right parietal and temporal lobes. The National Institute of Health in Washington diagnosed the vesicles as Cysticercus cellulosae, the larval form of Taenia solium.

GEORGE B. HASSIN.

SIDEROFIBROSIS OF THE SPLEEN IN SICKLE CELL ANEMIA. L. W. DIGGS, J. A. M. A. **104**:538, 1935.

This article is based mainly on a study of the spleen in nineteen cases of sickle cell anemia. The descriptions of the spleen in thirty-three cases of the disease reported in the literature are also considered. The various stages of the siderotic fibrosis of the spleen in sickle cell anemia are described in detail, but no conclusions are drawn as to the relationship of the splenic lesion to the severity and duration of the anemia. The specific feature is the presence in the spleen, in all stages of the process, of sickle-shaped red corpuscles. The process is the result of hemorrhages into the spleen followed by replacement with connective tissue in which are deposits of iron and calcium. In these deposits may occur structures that look more or less like mycelial bodies, but there is no convincing evidence that fungi play any part in the process.

ELLIPTICAL HUMAN ERYTHROCYTES. D. J. STEPHENS and A. J. TATELBAUM, J. Lab. & Clin. Med. **20**:375, 1935.

The authors present hematologic studies of fifteen members of an Italian family in which eight members exhibited elliptic erythrocytes. In each of the affected ones there was an increase in the number of red blood cells per cubic millimeter with a definite increase in the mean corpuscular volume and the mean corpuscular hemoglobin. In general the number of red blood cells was inversely proportional to the mean cell volume and the mean hemoglobin content. Two dimensional measurements of cells in fixed, stained smears indicated that the decrease in size involved the round cells and the abnormally shaped cells to approximately the same extent, without increase in the variability of distribution of cell size. In the unaffected members of the family the erythrocytes were normal in shape, size, volume and hemoglobin content.

FROM THE AUTHORS' SUMMARY.

RELATION OF ADENOMA OF THE UMBILICUS TO APOCRINE SWEAT GLANDS AND TO ADENOFIBROSIS OF THE MAMMARY GLAND. H. GEHRKE, Virchows Arch. f. path. Anat. **293**:191, 1934.

Gehrke reviews the theories of the pathogenesis of umbilical adenoma, for which he prefers the name "adenofibrosis," and of the endometrioid proliferations in gen-

eral. He finds little support for the view that the umbilical tumor arises from celomic epithelium or from metastatic endometrioid tissue. He describes the histologic appearance of an umbilical nodule from a woman aged 89 and attaches great importance to the myxomatous character of the stroma about the glandular elements. At the pheriphery of the tumor there occurred transitions from normal sweat glands to cysts and glands with columnar epithelium. In the transition zone were sweat glands of apocrine type with myxomatous stroma. He accepts as established the normal occurrence of apocrine sweat glands in the umbilicus of woman. It is from these that he derives the umbilical adenoma. Participation of such tumors in the menstrual cycle is not proof of their endometrial origin, since the apocrine sweat glands are influenced by sex hormones. In cystic disease or adenofibrosis of the mammary gland the presence of large glands of apocrine sweat gland type is frequently observed. The myxomatous character of the stroma about them is cited by Gehrke in support of his theory. O. T. SCHULTZ.

HISTOLOGY OF BONE INVOLVEMENT IN PARONYCHIA. G. A. SEDGENIDSE, Virchows Arch. f. path. Anat. **293**:207, 1934.

Two forms of involvement of the terminal phalanx in suppurative paronychia have been described: (1) periostitis and involvement of the subadjacent bone by direct continuity from the infected soft tissues and (2) osteomyelitis by way of the blood stream. Histologic examination of early lesions leads the author to conclude that such a difference does not exist. The terminal phalanges have a much richer blood supply by way of the periosteum than other bones. The inflammatory process attacks the bone by way of the periosteum; osteomyelitis may result as a late stage of this process but not as a primary process beginning in the medullary spaces of the bone. The entire phalanx may die early in the infection; sequestration of part of the bone does not occur because the rich blood supply of the periosteum permits rapid regeneration if the inflammation has not been so diffuse as to cause complete necrosis in a short time. O. T. SCHULTZ.

THROMBOPHILIA AND POLYCYTHEMIA. H. LÜDEKE, Virchows Arch. f. path. Anat. **293**:218, 1934.

The term "thrombophilia," according to Lüdeke, should be reserved for a condition characterized by a pathologic disposition to multiple, not infrequently recurring, thrombosis, often in vessels not usually the site of this process, in persons who do not exhibit the factors usually held responsible for thrombosis. Four cases are presented. Platelet counts were not made. In two cases histologic examination of the bone marrow revealed a great increase in megakaryocytes. In the other two, bone marrow was not available for study, but the clinical picture was that of polycythemia. Lüdeke holds that all were cases of polycythemia. He tabulates from the literature twenty-one additional cases of polycythemia with thrombosis. Thrombosis in polycythemia is due to increased formation and delivery into the blood of platelets (thrombocytosis) and to slowing of the circulation. The latter phenomenon is due to increased viscosity of the blood and to an increase in the cross-sectional area of the circulatory bed brought about by the increased blood volume. The localization of the thromboses depends on local peculiarities of the circulation. Polycythemia is a striking example of a pathologic predisposition to thrombosis. O. T. SCHULTZ.

ENDARTERIAL CELLULAR BUDS OF THE THYROID. A. GILPIN, Virchows Arch. f. path. Anat. **293**:257, 1934.

In 1892 Horne described in goitrous thyroids areas of cellular proliferation in the small arteries. They projected into the lumens of the vessels as small cellular buds. These findings were confirmed by M. B. Schmidt, who found the buds also in normal thyroids. Concerning their origin and nature there has been doubt.

Gilpin examined serial sections of fifty thyroids, normal and abnormal, from persons of various ages. He found the structures described by Horne. They were most numerous in the most highly vascularized thyroids. They are not true proliferations but artefacts resulting from a plane of section through twists and bends of the smaller arteries. The cellular nodules usually consist only of intima, but they may contain tissues of the other two coats as well. O. T. SCHULTZ.

THYROTOXIC MYOCARDIAL CHANGES. D. BOYKSEN, Virchows Arch. f. path. Anat. **293**:342, 1934.

Histologic examination of the hearts of twenty-five persons who died of thyrotoxicosis revealed myocardial changes in twenty-three. The detectable alterations were primarily regressive and began with loss of striation and degeneration of individual muscle fibers or small groups of fibers and proceeded to necrosis of myocardial fibers. The degenerative changes were associated with lymphocytic infiltration and with replacement of degenerated fibers by proliferated connective tissue. Causes of damage other than thyrotoxicosis were excluded. Similar changes were produced in rats subjected to subcutaneous administration of synthetic thyroxine. Myocardial damage was increased if the thyroxinized rats were exercised daily on a treadmill to exhaustion. Similar muscular activity in rats without thyroxine led to no myocardial changes. Boyksen holds that the myocardial damage of hyperthyroidism is due to thyrotoxic action on the muscle fibers.

O. T. SCHULTZ.

PATHOLOGIC ANATOMY OF HUMAN PSITTACOSIS. B. J. MANSENS, Nederl. tijdschr. v. geneesk. **78**:5818, 1934.

The lung may show lobular pneumonia with a gray or red, smooth, moist cut surface. The exudate consists mostly of serous fluid with little fibrin, some large mononuclear cells and few leukocytes. There may be fibrinous pleuritis. In the liver and kidney there is degeneration of the epithelium, and sometimes there is focal necrosis in the liver. There may be swelling of the reticulo-endothelial cells in the spleen and in the liver, also small hemorrhagic extravasations in different organs.

Pathologic Chemistry and Physics

THE BLOOD AND PLASMA GLUTATHIONE CONTENT IN HUMAN CANCER. J. W. SCHOONOVER, Am. J. Cancer **23**:311 and 315, 1935.

The average reduced and oxidized glutathione contents of the blood of patients with cancer is practically the same as in normal blood. The reduced glutathione content of erythrocytes is slightly higher than normal in the presence of both cancer and nonmalignant tumors, while that of blood plasma is low and fairly uniform. The ratio of oxidized to reduced glutathione is higher in the plasma and lower in the erythrocytes of patients with cancer than in normal persons.

ELIZABETH MCBROOM.

PHOSPHATASE IN OSSIFYING MYOSITIS. W. E. WILKINS, M. REGEN and G. K. CARPENTER, Am. J. Dis. Child. **49**:1219, 1935.

Sections of tissue from a patient with so-called myositis ossificans progressiva were studied for evidence of phosphatase activity. Taken from the region of two lesions in the preossification stage in a patient with progressive ossifying myositis, fibrous tissue had a much higher phosphatase activity than muscle. The activity in two samples of normal muscle was low. Samples of heterotopic bone and cartilage from an older lesion had a higher activity than a piece of normal rib.

FROM THE AUTHORS' SUMMARY.

CALCIUM-ION CONCENTRATIONS IN THE BLOOD. F. C. McLEAN and A. B. HASTINGS, Am. J. M. Sc. **189**:601, 1935.

The total calcium of the serum or plasma is nearly all accounted for as calcium ions and calcium bound to protein. Of these two forms, the ionized calcium is of primary physiologic and clinical importance. The concentration of calcium ions in the plasma is normally maintained within a relatively narrow range by a process of physiologic regulation in which the parathyroid glands play a prominent part. This range has been found to be, as a rule, from 4.25 to 5.25 mg. per hundred cubic centimeters. The concentration of calcium ions in the plasma at any time is the resultant of an equilibrium between the total calcium and the total protein in the plasma. A method for clinical estimation of calcium ion concentration by calculation from the total protein and the total calcium concentration is presented. Results obtained by this method have been found to be in substantial agreement with those obtained by direct observation. Fluctuations in the concentration of total calcium in response to fluctuations in that of total protein in the plasma are necessary for the maintenance of a normal calcium ion concentration, and consequently are not in themselves of clinical importance. An increase in the concentration of calcium ions in the plasma is presumptive evidence of hyperfunction of the parathyroid glands. A decrease in the concentration of calcium ions in the plasma may occur as the result of hypofunction of the parathyroid glands, or may be brought about by the hyperphosphatemia of uremia. In all other conditions investigated, including various disorders of calcification, the concentration of calcium ions in the serum or plasma has been found to be within normal limits.

FROM THE AUTHORS' SUMMARY.

THE LIPID CONTENT OF LIVERS OF NON-IMMUNIZED AND IMMUNIZED HORSES. A. WADSWORTH, L. W. HYMAN AND R. R. NICHOLS, Am. J. Path. **11**:419, 1935.

The lipids in the liver of immunized and those of nonimmunized horses were determined by estimating the total fatty acids, phospholipids, free and esterified cholesterol and the iodine value of the total fatty acids. The values for the immunized showed much wider variation than did those for the normal animals. The ratio of phospholipid to free cholesterol in the liver from the immunized horses was lower than in that from the nonimmunized ones and corresponded, in general, to the changes in the liver.

GASTRIC ACIDITY IN PULMONARY TUBERCULOSIS. I. GRAY and J. MELNICK, Am Rev. Tuberc. **31**:460, 1935.

Of young persons between 20 and 30 years of age with pulmonary tuberculosis of from one to six or more years' duration, approximately one third presented gastric hypo-acidity and one fourth anacidity within a period of from one to four years. Approximately one half of the number showed a normal acidity at all times. Of male patients, a little more than half showed normal acidity through a six year period of the disease. Approximately one third showed a decrease or an absence of acid. Of female patients only one third had normal acidity, the other two thirds showing a decrease or an absence of acid. There was a definite tendency in the females toward a decrease in acid. The gastric contents of the majority of the young adults showed tubercle bacilli. As the acid decreased tubercle bacilli were found more frequently in the stomach contents. Gastric symptoms were somewhat more frequent in the females than in the males and usually started at an earlier period.

H. J. CORPER.

RELATION OF PLASMA PROTEINS TO ASCITES AND EDEMA IN CIRRHOSIS OF THE LIVER. W. K. MEYERS and C. S. Keefer, Arch. Int. Med. **55**:349, 1935.

Sixteen cases of cirrhosis of the liver were studied. A deficiency of total plasma protein was found. The decrease was most pronounced in the albumin

fraction. The albumin-globulin ratio was inverted. Similar but less extensive and less consistent alterations in the values of plasma protein were observed in fourteen cases of other forms of disease of the liver.

In patients with cirrhosis of the liver the following conditions were observed: 1. The protein content of the ascitic fluid varied between 0.1 and 1.7 Gm. per hundred cubic centimeters. The albumin content of the ascitic fluid was proportionately greater than that of the blood plasma. 2. The removal of ascitic fluid gave rise to changes in the plasma protein value and temporary hydremia with corresponding diuresis. 3. Peripheral edema without circulatory failure was associated with a reduction of the plasma proteins.

In cirrhosis of the liver the appearance of ascites must depend somewhat on the osmotic pressure of the blood plasma as well as on portal obstruction. The hypoproteinemia may arise from defective formation of plasma proteins and from loss of protein into the ascitic fluid. The defect may be due to alteration in the function of the liver. FROM THE AUTHORS' SUMMARY.

THE UREA RATIO AS A MEASURE OF RENAL FUNCTION. H. O. MOSENTHAL and M. BURGER, Arch. Int. Med. **55**:411, 1935.

The urea ratio expressed as the percentage of urea nitrogen in the total nonprotein nitrogen of the blood, or $\frac{100 \times \text{urea nitrogen,}}{\text{nonprotein nitrogen}}$ is a satisfactory index of renal efficiency. When the Folin-Wu filtrate (laked blood) is used for the determination of both the urea nitrogen and the total nonprotein nitrogen in the blood it is noted that the urea ratio varies with renal function as follows: (*a*) With normal renal function the index is 44 or less; (*b*) with maximal impairment of renal function the index is 80 or higher; (*c*) with improvement in renal function, a drop in the ratio, and with progressive impairment of function, a rise in the ratio occurs. The determination of the urea ratio at intervals measures progressive changes in renal efficiency and affords a reliable index of renal function regardless of the fluctuations of the total nonprotein nitrogen of the blood. Although the urea nitrogen or the total nonprotein nitrogen in the blood may be at a normal level, the urea ratio reveals renal insufficiency when it exists. This test may be carried out on one specimen of blood; it does not require prolonged observation of the patient or collection of urine. It furnishes a numerical index of impairment of renal function and is equally applicable to patients in private practice and to those in hospitals. FROM THE AUTHORS' SUMMARY.

ALKALI RESERVE IN BLOOD AND IN CEREBROSPINAL FLUID IN EXPERIMENTAL ACIDOSIS. E. DE THURZO and S. KATZENELBOGEN, Arch. Neurol. & Psychiat. **33**:786, 1935.

The authors studied the blood (from the heart) and the spinal fluid (obtained by cisternal puncture) of cats for carbon dioxide-combining power before and after the blood had been rendered acidotic by intraperitoneal injections of ammonium chloride (from 0.25 to 1 cc. of 10 per cent solution). In some cats, in order to increase the permeability of the barrier between the blood and the spinal fluid, aseptic meningitis was produced before the intraperitoneal injection of ammonium chloride. The carbon dioxide-combining power was studied at various intervals after injection of ammonium chloride. In eight of twenty-five experiments, acidosis of the blood was generally accompanied by a mild reduction in the carbon dioxide-combining power of the cerebrospinal fluid. This was much more marked in animals in which aseptic meningitis had been additionally produced, so that the acid-base balance in the cerebrospinal fluid is, in the authors' opinion, apt to be affected by a combination of acidosis of the blood and an inflammatory process within the cerebrospinal cavity. GEORGE B. HASSIN.

URINARY SILICA IN PERSONS EXPOSED TO SILICA DUST. J. J. BLOOMFIELD, R. R. SAYERS and F. H. GOLDMAN, Pub. Health Rep. **50**:421, 1935.

One hundred and twenty-three anthracite coal workers, twenty of whom had been out of the industry an average of seven years, were examined for urinary silica by the method of King and Dolan. The amounts of silica found in the urine varied from 0.6 to 11.7 mg. and averaged 2.5 mg. per hundred cubic centimeters. Normal persons were found to be excreting only an average of 1 mg. per hundred cubic centimeters. A close correlation was found between the exposure of these men to silica dust for a specified number of years and the amount of urinary silica. A study of former anthracite coal workers showed that even after a lapse of several years away from any exposure to silica dust an increased amount of silica is being excreted by them. These findings furnish additional evidence of the etiology of the disease.

FROM THE AUTHORS' SUMMARY.

THE LIPIDS IN THE LEUKOCYTES IN FEVER AND INFECTION. ELDON M. BOYD, Surg., Gynec. & Obst. **60**:205, 1935.

The leukocytes undergo marked metabolic variations in pregnancy, lactation, postoperative convalescence and fever, as indicated by changes in their lipid content. There are twenty-six complete differential analyses of lipids in the leukocytes under fasting conditions in twelve cases of fever. In each analysis the lipids determined were: free cholesterol, ester cholesterol, total cholesterol, phospholipid, neutral fat, total fatty acids and total lipid. Oxidative micromethods were used. The results are grouped according to the outcome of the infections. In patients who recovered from fever due to a variety of causes the white blood cells contained large amounts of phospholipid. There was generally also an increase in free cholesterol with low figures for ester cholesterol and neutral fat. In these, convalescence was accompanied by a further rise in phospholipid. Patients dying from infection had low phospholipid values in the blood leukocytes during the febrile period. It appears that the activity of the leukocytes may be measured by their lipid content, especially by the phospholipid fraction.

WARREN C. HUNTER.

HAEMOCHROMATOSIS. H. RAMAGE and J. H. SHELDON, Quart. J. Med. **4**:121, 1935.

In hemochromatosis there is an increase of iron in all the tissues of the body with the exception of the blood, brain and colon; concerning the latter two organs, however, the statement is subject to individual exceptions. In certain organs the amount may be enormous, especially in the liver, pancreas, lymph glands, thyroid, salivary glands, pituitary, choroid plexuses and heart. The increase over normal appears to be greatest in the pancreas. The somatic muscles share in these deposits of iron. The total amount of iron deposited in the body by the time of death appears to vary from about 25 Gm. to from 45 to 50 Gm. There is a slight increase of the total sulphur in certain tissues, especially in the alimentary canal. This is probably related to the deposits of hemofuscin. Spectrographic examination confirmed the results of previous chemical analyses in showing that there is an increase of copper in the liver. This increase applies to all the tissues with the exception of the kidney, small intestine and omentum. The general order of increase is between two and three times the normal. Certain of the tissues have an increase of calcium, which is best seen in the liver, thyroid, striated muscles and pancreas. Most of the tissues show disturbances in the behavior of both sodium and potassium, these metals usually swinging in opposite directions. The manganese content of the liver is about one-fourth the normal. No unusual elements were met with.

FROM THE AUTHORS' SUMMARY.

Microbiology and Parasitology

AN INTERPRETATION OF THE FILTERABLE VIRUSES. R. R. HYDE, Am. J. Hyg. **21**:472, 1935.

"A filterable virus, as we conceive it today, is an agent of particulate nature. It is of ultramicroscopic dimensions. It passes filters that retain the vegetative forms of parasitic life. It can be passed in series on sensitive hosts. It produces characteristic inclusion bodies. It has not been cultivated on lifeless media. It would be unknown to us were it not for the changes produced in the bodies of higher animals and plants."

RELATIONSHIP BETWEEN RHEUMATIC AND SUBACUTE BACTERIAL ENDOCARDITIS. W. C. VON GLAHN and A. M. PAPPENHEIMER, Arch. Int. Med. **55**:173, 1935.

The infection of the cardiac valves with nonhemolytic streptococci in rheumatic patients is due to implantation of the bacteria on unhealed rheumatic vegetations. The continued activity of the rheumatic disease in patients with subacute bacterial endocarditis is attested by the invariable occurrence on the valves of rheumatic verrucae which do not contain bacteria, and by Aschoff bodies in the myocardium in the same percentage of cases as in uncomplicated rheumatic carditis. Staphylococcus aureus may also be implanted on unhealed rheumatic vegetations.

FROM THE AUTHORS' CONCLUSIONS.

THE FIBRINOLYTIC ACTIVITY OF HEMOLYTIC STREPTOCOCCI. W. S. TILLETT, J. Bact. **29**:111, 1935.

There is a relationship between the infectivity of the beta hemolytic streptococcus for man and the fibrinolytic property of the cultures. The determination of the presence or absence of fibrinolysin in the cultures is helpful in separating strains pathogenic for man from others which probably do not cause disease in human beings.

FROM THE AUTHOR'S CONCLUSIONS.

RABBIT POX. H. S. N. GREENE, J. Exper. Med. **60**:427 and 441, 1934.

The clinical manifestations and course of a highly contagious and acutely fatal disease designated as rabbit pox have been described. The general symptomatology varied within wide limits, and accurate diagnosis rested on the occurrence of certain characteristic lesions. The most distinctive of these lesions was a pocklike eruption which was often outspoken and widespread over the body, but sometimes was poorly defined and detectable only after careful physical examination. Other lesions depended on involvement of particular organs and gave rise to special symptoms. As a rule, in the epidemic infection the period of incubation varied between five and seven days. No evidence of transmission of the infection by animals which had recovered could be obtained. Furthermore, tests demonstrated that the reproductive capacities of most of the animals were not permanently impaired.

The most distinctive gross lesion in all organs and tissues was the small nodule or papule, which was found to consist of mononuclear infiltration and necrosis. Diffuse lesions were also found in which the infiltration was widespread and accompanied by edema, hemorrhage and extensive necrosis of the affected tissues and organs. The possibility that the diffuse lesions were due to the action of secondary invaders was considered, but the available evidence indicated that the different types, including pneumonia, represented reactions to a single causative agent. Moreover, an intimate relationship was observed to exist between lesions and small blood vessels, in which primary endothelial damage was usually apparent. The degree of vascular damage generally corresponded to the extent of the

lesion, and it is probable that this in turn corresponded to the dose of the causative agent. The close analogy between the clinical manifestations and pathologic processes of this disease and smallpox in man led to the conclusion that the disease in the rabbit is essentially the same as smallpox, and that it is probably produced by a virus closely related to that of smallpox. The available evidence indicated that the infection originated in the institute (Rockefeller Institute for Medical Research) and that it spread in atypical form or masked by some other disease until it reached the breeding colony as a clearly defined epidemic infection.

FROM THE AUTHOR'S SUMMARIES.

TRANSMISSION OF KALA-AZAR BY ORONASAL SECRETIONS. C. E. FORKNER and L. S. ZIA, J. Exper. Med. **61**:183, 1935.

Patients with kala-azar, whether the symptoms of the disease are of short or long duration, almost without exception have present in their oral and nasal discharges viable, pathogenic Leishmania Donovani. Evidence is presented which strongly supports the theory that kala-azar is transmitted by means of direct or indirect contact. Unequivocal proof of the important natural mode or modes of transmission of kala-azar has not yet been presented. Much more work must be done before a final solution of the problem can be accepted.

FROM THE AUTHORS' CONCLUSIONS.

DIAGNOSIS OF PSITTACOSIS IN MAN BY MEANS OF INJECTIONS OF SPUTUM INTO WHITE MICE. T. M. RIVERS and G. P. BERRY, J. Exper. Med. **61**:205, 1935.

By means of intraperitoneal injections of unfiltered sputum or of filtrates of sputum into mice, psittacosis in human beings can be successfully diagnosed in a large percentage of the cases and in a relatively safe manner.

FROM THE AUTHORS' SUMMARY.

REPEATED ATTACKS OF EXPERIMENTAL PNEUMOCOCCUS LOBAR PNEUMONIA IN DOGS. L. T. COGGESHALL and O. H. ROBERTSON, J. Exper. Med. **61**:213, 1935.

A study has been made of repeated attacks of lobar pneumonia produced in dogs by intrabronchial injection of Pneumococcus, type I. Twenty-five dogs were given seventy-eight infections at intervals of from three days to nineteen months. The number of attacks to which a single animal was subjected varied from two to eleven. It was found that recovery from this experimental disease conferred on the animals increased resistance against subsequent infections, as shown by the fact that such animals regularly survived doses of culture which in dogs infected for the first time produced a fatal outcome. The recurrent attacks of pneumonia were uniformly mild, the febrile course was brief, the pulmonary lesion was usually confined to a single lobe, and bacteremia seldom occurred. There was no detectable difference between the second and the subsequent infections, which could be produced whenever desired, nor did the intervals of time between attacks appear to bear any relation to the severity of the experimental disease. Tests for acquired antipneumococcic immune substances in the blood after recovery showed that these substances were present in some animals and not in others, yet dogs without demonstrable humoral immunity appeared to be just as resistant to reinfection as those possessing it. A comparison of the pathogenesis of these secondarily induced lesions with that of the initial infection revealed certain striking differences. Secondary lesions produced in a lobe previously affected tended to evolve much more rapidly than did primary ones. They were characterized by early appearance of a generalized macrophage reaction and marked diminution in the numbers of pneumococci in the tissues or their complete absence. These changes occurred more slowly in secondary lesions initiated in hitherto uninvolved lobes. The macrophage reaction, which consists of a swelling of the fixed tissue cells (histiocytes) and a

subsequent liberation of macrophages into the alveolar exudate, is regarded as significant evidence of increased antipneumococcic resistance, since it has been observed to occur regularly at the time of recovery from the first infection and is accompanied by local disappearance of the invading micro-organisms.

FROM THE AUTHORS' SUMMARY.

CORYZA OF THE DOMESTIC FOWL. J. B. NELSON, J. Exper. Med. **61**:351 and 361, 1935.

The mucous surfaces of the nasal passages and orbital sinuses appear to afford particularly favorable conditions for the development of the bacillus of fowl coryza. Injected into the nasal tract in any appreciable number, the bacilli regularly develop and may continue to exist for a considerable period in spite of an active inflammatory reaction on the part of the host. The bacillus multiplies either sparsely or not at all when injected extranasally, regardless of the nature of the cellular surface with which it is brought in contact. If the locus of injection is in communication with the upper air passages, as in the instance of the trachea, internal ear and orbital cavity, the bacilli may be carried there even in the absence of a local development and produce coryza. Introduction of the bacilli in loci not in communication with the upper air passages is followed by nasal carriage only in the case of the peritoneal cavity. Following intraperitoneal injection of the bacilli, seven of twelve birds showed these organisms in the nasal passages and, except in one instance, without accompanying inflammation.

Intratracheal, intracloacal and subcutaneous injection of living cultures of the bacillus of fowl coryza had no demonstrable effect on the susceptibility of fowl to coryza. Intraperitoneal injection was irregularly followed by definitely altered susceptibility. Growth of the specific bacillus was inhibited in the nasal tracts of approximately 70 per cent of twenty-five birds which had received an earlier intraperitoneal injection.

FROM THE AUTHOR'S SUMMARIES.

THE COLONY MORPHOLOGY OF TUBERCLE BACILLI. K. C. SMITHBURN, J. Exper. Med. **61**:395, 1935.

Smooth, round, shiny, nongranular and nonspreading colonies have been observed in cultures of virulent tubercle bacilli freshly isolated from eight human sources other than sputum. The classification of six of these strains as of human type was established by inoculation of the cultures into rabbits and guinea-pigs. The 3 per cent sodium hydroxide or 6 per cent sulphuric acid frequently used in the isolation of tubercle bacilli are definitely unfavorable to the development of smooth colonies. It was observed that smooth colonies are produced in greater number when the hydrogen ion concentration of the medium (Corper's egg yolk-glycerin medium) is adjusted to a point near to but slightly on the acid side of neutral.

FROM THE AUTHOR'S SUMMARY.

EXPERIMENTAL STUDIES ON ENCEPHALITIS. L. T. WEBSTER and G. L. FITE, J. Exper. Med. **61**:411, 1935.

The infectious agent in fatal cases of St. Louis and Kansas City encephalitis passes Seitz pads in high dilution without appreciable loss of infectivity and traverses collodion membranes with an average pore size of 66 microns or greater. It is highly infectious for mice by intracerebral and intranasal routes but practically innocuous by subcutaneous and intraperitoneal routes. Certain strains of mice are more susceptible than others. Administered to mice intranasally, the agent causes tremors and convulsions after an incubation period of from six to seven days, followed by prostration and death in from eight to ten days. Lesions are demonstrable in the olfactory bulbs three days after infection, in the pyriform lobe after four days, and in Ammon's horn after five days. In monkeys (Macacus rhesus) the agent provokes a mild, nonfatal reaction and the development of specific neutral-

izing bodies. On passage in monkeys the virus becomes progressively weaker. In rabbits, guinea-pigs, rats and sheep it is apparently without effect. All available strains proved alike in their effects on animals and in immunologic respects. The available data enable one to conclude that the agent is a filtrable virus differing from those studied heretofore.

FROM THE AUTHORS' CONCLUSIONS.

IS A SPECIAL VARIETY OF STAPHYLOCOCCUS CONCERNED IN FOOD POISONING? J. STRITAR and E. O. JORDAN, J. Infect. Dis. **56**:1, 1935.

As other investigators have found, there are no good criteria for the differentiation of various types of staphylococci. Biochemical, hemolytic and agglutinative characters are not closely correlated, and except for differences in the vigor of the strains it is hardly possible to maintain a sharp distinction between the albus and aureus varieties or between strains of "saprophytic" and those of pyogenic origin. The "food poisoning" strains agree with the other members of the group in not constituting a clearly marked division. There is no evidence of homogeneity in biochemical, hemolytic or agglutinative characters. The power to provoke food poisoning is not limited to any recognizable variety of staphylococci. As bearing on the possible instability of these organisms it is worth noting that strains with different biochemical and serologic characters have been isolated from the same article of food (e. g., cake) in one and the same outbreak (cf. strains 1, 3, 4, table 1). On the other hand, the unity of the strains found in some outbreaks (e. g., 142-5) is noteworthy.

FROM THE AUTHORS' SUMMARY.

EXPERIMENTAL RABIES IN WHITE MICE AND ATTEMPTED CHEMOTHERAPY. A. HOYT, R. T. FISK and C. H. THIENES, J. Infect. Dis. **56**:21, 1935.

Groups of white mice, each comprising approximately twenty-four animals, were given intracerebral injections of fixed rabies virus. They were subsequently treated with various drugs in an attempt to prevent or modify the course of the disease. The drugs employed were plasmochin, merthiolate, metaphen, bismuth violet, iodobismitol, bismarsen, tryparsamide, silver arsphenamine, Bayer 205, ethylhydrocupreine hydrochloride (optochin), pyridium, sodium arsanilate (atoxyl), neostam and sparteine sulphate. The virus and the drugs were injected in amounts directly proportional to the body weight of each mouse. The experiments performed were divided into sixteen series and an adequate control group of mice was instituted in each series. The mean period of incubation and the mean length of life (after injection of fixed virus) were calculated for each group of mice. Neither of these periods differed significantly as between the treated and control groups of mice included in any one series of experiments.

FROM THE AUTHORS' SUMMARY.

THE PASSAGE OF BACTERIA FROM THE LUNGS INTO THE BLOOD STREAM. W. M. TUTTLE and P. R. CANNON, J. Infect. Dis. **56**:31, 1935.

Suspensions of cultures of Staphylococcus aureus, Bacillus prodigiosus and Streptococcus haemolyticus were introduced into the lower lobe of the left lung in fourteen healthy dogs, and samples of blood from the femoral artery and lymph from the thoracic duct were cultured at frequent intervals during the first sixty minutes. In the animals given injections of cultures of Staph. aureus and B. prodigiosus both the blood and lymph remained sterile throughout the period of observation. The bacteria were demonstrable in the lungs at the end of the experiment, many engulfed by septal cells. In the dogs given injections of cultures of the hemolytic streptococcus, however, the micro-organisms quickly entered the blood stream whereas the thoracic lymph remained sterile in most instances, proving that the passage of bacteria from the lungs into the blood stream was direct rather than indirect by way of the lymphatics. It is probable that the differences in the response of the pulmonary tissues to the micro-organisms employed depended to

a large extent on the varying degrees of injury to the cellular and tissue barriers within the lungs and that the experimental bacteremia occurred because of increased permeability of these membranes induced by the virulence and toxigenicity of the bacteria.

FROM THE AUTHORS' SUMMARY.

THE SPECIFICITY OF THE CONJUNCTIVAL INFECTION IN MONKEYS FOLLOWING INOCULATION WITH TRACHOMATOUS TISSUE. R. W. HARRISON, J. Infect. Dis. **56**:49, 1935.

It has not been possible to induce folliculosis in monkeys nonspecifically by mechanical or chemical irritation. Follicles have not appeared after inoculation with a variety of bacteria isolated from patients with trachoma and other ocular diseases. Repeated inoculations with bacteria and protein solutions continued long enough to establish hypersensitive responses have failed to induce follicle formation. Inoculation with tissue from patients with acute conjunctivitis, spontaneous folliculosis and inactive trachoma failed to elicit a follicular response in monkeys. In animals recovered from infection by inoculation with trachomatous tissue, follicular hypertrophy was induced only by reinoculation with trachomatous material. Monkeys which did not react to nonspecific stimuli subsequently acquired typical follicles when inoculated with active trachomatous material. These findings indicate that the experimental disease in monkeys is specific for trachoma.

FROM THE AUTHOR'S SUMMARY.

COCCUS FORMS OF CORYNEBACTERIUM DIPHTHERIAE. T. C. GRUBB, J. Infect. Dis. **56**:64, 1935.

An irregular alternation between the appearance of bacillary and that of coccus forms of the diphtheria bacillus was found when it was aged or repeatedly transferred in various mediums. A small colony variant, or "G" colony, was observed which did not appear to be related to a life cycle or to the production of coccus forms. Coccus forms of certain strains were produced at will by growing the strains in liver infusion mediums. These cocci reverted to bacilli when transferred to favorable mediums. A direct microscopic observation on the warm stage was made of the bacillus-to-coccus and coccus-to-bacillus change. The virulence, toxigenesis and fermentation reactions of the coccus forms could not be satisfactorily determined. The differentiation of diphtheria and diphtheroid strains on liver infusion agar was not cleancut enough for diagnostic purposes. When methods employed by other workers for producing coccus forms were successfully duplicated, the resulting cocci were morphologically indistinguishable from those produced in liver infusion mediums. The addition of glycogen, dextrose or bile to veal infusion broth did not induce the formation of cocci.

FROM THE AUTHOR'S SUMMARY.

THE PATHOGENICITY FOR CATTLE OF BRUCELLA STRAINS FROM UNDULANT FEVER IN MAN. R. R. BIRCH and H. L. GILMAN, J. Infect. Dis. **56**:78, 1935.

Strains of Brucella isolated from patients with typical undulant fever proved regularly to be pathogenic for cattle, producing in them a sydrome indistinguishable from that which occurs in natural cases of Brucella infection otherwise known as infectious abortion.

FROM THE AUTHORS' CONCLUSION.

THE SPREAD OF AMEBIC DYSENTERY BY WATER. A. V. HARDY and B. K. SPECTOR, Pub. Health Rep. **50**:323, 1935.

During the stockyards fire in Chicago in May 1934 water heavily polluted with fresh sewage was used for drinking purposes by many firemen and spectators. There followed a large but undetermined number of cases of acute diarrhea, sixty-nine of typhoid fever and two of paratyphoid fever. Laboratory studies revealed

that a high percentage of those exposed had become infested with Endamoeba histolytica, and the more severe the symptoms, the higher the percentage. Six cases of undoubted amebic dysentery, two complicated with abscess of the liver, were recognized among those exposed. Six other mild diarrheas (one in a fireman and five in spectators) were also diagnosed as amebic dysentery and reported to the board of health. The evidence has led to the opinion that many of the other illnesses with intestinal symptoms were also the result of E. histolytica invasion.

Therefore it is concluded that infestations with E. histolytica may occur in association with water-borne epidemic diseases, and, furthermore, that the control of amebic dysentery demands that water for human consumption be free from dangerous protozoal as well as from bacterial contamination.

FROM THE AUTHORS' SUMMARY.

THE EXPERIMENTAL PRODUCTION OF MUMPS IN MONKEYS. G. M. FINDLAY and L. P. CLARKE, Brit. J. Exper. Path. **15**:309, 1934.

Bacteriologically sterile saliva taken from a patient with mumps forty-eight hours after the onset and injected directly into Stensen's duct in rhesus monkeys produces a mild febrile reaction, swelling of the parotid glands and histologic changes very similar to those seen in human mumps. There is also a leukopenia. The virus has been passed through monkeys six times. It can be preserved in 50 per cent glycerin for at least five weeks. Injection of the virus into the tunica vaginalis of a monkey causes a nonsuppurative orchitis. Mice, rats and guinea-pigs inoculated intracerebrally did not present any symptoms.

AUTHORS' SUMMARY.

THE EXPERIMENTAL B. VIRUS DISEASE IN MACACUS RHESUS MONKEYS. A. B. SABIN, Brit. J. Exper. Path. **15**:321, 1934.

The B virus is pathogenic for rhesus monkeys and has been transmitted through four generations by intracerebral inoculation. Intracerebral inoculation of the virus is usually fatal, and gives rise to various clinical syndromes. The most striking features of the disease following intravenous inoculation of the virus are an exanthem (chiefly about the forehead, eyes and face) and an enanthem (in the buccal mucous membranes, tongue, palate, conjunctivae) which closely resemble the picture produced by intravenous inoculation of vaccinia in rhesus monkeys. Intracutaneous injection of the virus is followed by acute inflammation with necrosis, which may be hemorrhagic or vesiculopustular. Macroscopically the lesion bears some resemblance to that produced by vaccinia, but microscopically it is readily differentiated from the latter by the presence of acidophilic intranuclear inclusions in the epithelial cells and by the absence of Guarnieri bodies. Intraperitoneal injection of the virus gives rise to acute peritonitis with occasional invasion of some of the abdominal viscera. In monkeys peripheral inoculation of the virus does not lead to any apparent involvement of the central nervous system, and in this respect the virus lacks one of its most striking and constant properties in rabbits. Among 13 "normal" monkey serums, only one was found to contain antibodies for the B virus, and the titer of this one was as high as that of the best hyperimmune serum; on this basis it is believed that the monkey may at one time have had a natural B virus infection. Similarly, the opinion is expressed that the reason for the failure to establish this virus in rhesus monkeys in New York is that the majority of them were immune, probably as a result of natural infection, and that the human disease from which the B virus was isolated was caused by the bite of a monkey which was infected with it.

FROM THE AUTHOR'S SUMMARY.

ENCEPHALOMYELITIS PRODUCED BY NEUROTROPIC YELLOW FEVER VIRUS. G. M. FINDLAY and R. O. STERN, J. Path. & Bact. **40**:311, 1935.

The neurotropic yellow fever virus produced in susceptible animals (*a*) an inflammatory reaction in the central nervous system, (*b*) degenerative changes in the nerve cells and (*c*) acidophilic intranuclear inclusions. The inflammatory reaction was characterized by infiltration with mononuclear cells and proliferation of the microglia. Degenerative changes in the ganglion cells were present in all stages and ranged from slight swelling of the cell body to neuronophagia with complete disintegration of the cell. Specific acidophilic intranuclear inclusions were seen only in ganglion cells which had not undergone extensive degeneration and never in cells exhibiting neuronophagia. The inclusions did not stain with the Feulgen technic. Demyelination was nowhere observed.

FROM THE AUTHORS' CONCLUSIONS.

DIFFERENTIATION OF STREPTOCOCCUS PYOGENES FROM MAN AND ANIMALS BY THE SORBITOL-TREHALOSE TEST. F. C. MINETT, J. Path. & Bact. **40**:357, 1935.

Examination of a series of strains of Str. pyogenes from man and from cow's milk has given support to the view that strains of this kind from man are trehalose fermenters, while those from cow's milk are ordinarily sorbitol fermenters. Considered in conjunction with the work of previous observers, the sorbitol-trehalose test thus appears to be a simple method of establishing the original source of Str. pyogenes when present in milk. Apart from Streptococcus equi, ten of twelve strains isolated from equines fermented sorbitol and not trehalose. Judged by the methylthionine chloride reduction test, however, the two remaining strains were probably of animal type. Eleven of the twelve strains from dogs, cats and ferrets fermented neither sorbitol nor trehalose but differed from Str. equi in fermenting lactose. Attention is again called to a group of streptococci found in milk which ferment both trehalose and sorbitol. These streptococci, however, although they are actively hemolytic and fail to split sodium hippurate, should be excluded from the Str. pyogenes group.

FROM THE AUTHOR'S SUMMARY.

Tumors

A REVIEW OF THE RECENT LITERATURE OF TAR CANCER (1927-1931 INCLUSIVE). M. G. SEELIG and Z. K. COOPER, Am. J. Cancer **17**:589, 1933.

Our purpose, in this paper, is to bring, within convenient and compact form, the literature of tar cancer up to the beginning of 1932. William H. Woglom (ARCH. PATH. **2**:533, 1926) collected and abstracted the literature of tar cancer from the epochal work of Yamagiwa and Itchikawa in 1914 down to the end of 1926. Woglom's work not only constitutes a practical lexicon for all workers in tar cancer, but also makes it imperative that the encyclopedic topic of tar cancer be brought down to date, however trying and uninspiring may be the task of collective abstracting.

AUTHORS' SUMMARY.

MULTIPLE MYELOMA. E. SCOTT, F. M. STANTON and M. OLIVER, Am. J. Cancer **17**:682, 1933.

To the 425 cases of multiple myeloma already collected from the literature by Geschickter and Copeland, we have added 30 cases from the recent literature and 5 cases of plasma cell multiple myeloma occurring in our series of autopsies. . . . From a study of maturation stages in fast-growing myelomas and their similarity to experimentally produced plasma cells, we consider it probable that the tumor plasma cell is a derivative of the reticular cell of the hematopoietic and general connective tissues and is closely related to the lymphocyte series.

AUTHORS' SUMMARY.

GENETIC STUDIES ON THE TRANSPLANTATION OF TUMORS. J. J. BITTNER, Am. J. Cancer **17**:699, 709, 717 and 724, 1933.

The tumors inoculated in these experiments arose spontaneously and independently of each other in a single host. This mouse, no. 19308, was an F_1 hybrid animal produced by mating individuals of two highly inbred stocks of mice, the albino and the dilute brown. All tumors were diagnosed as adenocarcinomas of the mammary gland. They were termed 19308 A, B and C and were inoculated simultaneously into individuals of the two parental stocks and their reciprocal F_1, F_2 and back-cross generations. The total number of mice inoculated was 4,375.

Mice of the parental stocks gave negative results in grafts of tumor 19308 A, whereas in the F_1 generation 99.6 per cent of the animals inoculated were susceptible. The respective reciprocal hybrid generations gave differences which were not mathematically significant; the F_2 generation, observations which, when compared with expectations, showed that tumor 19308 A required the simultaneous presence of eight dominant mendelian factors for progressive growth, one of which was linked with the dilution color factor. Five of the susceptibility factors were derived from the dilute brown parental stock and three from the albino parental stock.

Tumor 19308 B grew progressively in all mice having the seven dominant mendelian factors necessary for susceptibility. One of the factors was linked with the dilution color factor. Three of the factors were contributed by the albino-race. The number derived from the dilute brown stock was between four and five. Two dilute brown males (litter-mates) grew tumor 19308 B.

Tumor 19308 C also required seven dominant factors for susceptibility, one of which was linked with the dilution color factor. Two or three of the susceptibility factors were derived from the albino stock and between four and five from the dilute brown stock.

Since tumors 19308 A, B and C were transplanted simultaneously, the observations were compared and an attempt was made to determine the number of susceptibility factors common to all three tumors. This number was probably five.

The value of the transplantation of neoplastic tissue still remains to be determined. It does show, however, that, on the simultaneous inoculation of multiple tumors from a single host, the physiologic and genetic constitution of some tumors is not identical with that of the host which gave rise to them.

Data secured from the transplantation of neoplastic tissue may aid in understanding the problem of cancer if one assumes that: (1) the etiologic factors may be due to hereditary or environmental causes; (2) cancer, if inherited, may be explained according to multiple dominant factors; (3) the physiologic characteristics of each cancer are determined by its genetic constitution, and (4) multiple neoplasms from a single host may be the same histologically but differ genetically and physiologically.

AUTHOR'S SUMMARY.

ON THE AMBIVALENT EFFECT OF A SINGLE ACUTE TRAUMA ON THE GROWTH OF TRANSPLANTED MOUSE TUMORS. M. C. MARSH, Am. J. Cancer **17**:735, 1933.

Sixty-three transplanted mouse tumors were crushed by one sudden pressure, and their subsequent growth was frequently measured. A sharp difference appeared between the reactions of smaller and larger tumors. The twenty-nine tumors that were 4 mm. or less in diameter were not inhibited to a significant degree. The thirty-four tumors above 4 mm. were stimulated in a degree that is probably statistically significant. When all increments of growth were combined and averaged the stimulation was nominal. There were seventy-one control tumors.

AUTHOR'S SUMMARY.

EMBRYONAL NEPHROMA IN A SHEEP. W. H. FELDMAN, Am. J. Cancer **17**:743, 1933.

The occurrence of an embryonal type of renal tumor in an 8 months' old sheep is reported. The tumor, which is designated as an embryonal nephroma, had

metastasized to the sublumbar lymph nodes and to the lungs. Although this type of neoplasm is not uncommon in chickens and swine, and has been reported in rabbits and in cattle, it apparently has not been reported previously in sheep.

AUTHOR'S SUMMARY.

ABSORPTION OF THE PROTECTIVE AGENT FROM RATS RESISTANT TO A TRANSPLANTABLE SARCOMA. WILLIAM H. WOGLOM, Am. J. Cancer **17**:873, 1933.

Frozen and thawed rat sarcoma 39 which has lain for a few days in the subcutaneous tissues of an immune rat accumulates from them a substance which is not present in normal rats, and then exerts an inhibitory effect on the growth of this neoplasm when mixed with it prior to inoculation. As the cells of R 39 are killed by freezing and thawing, it may be fairly said that the suppressing agent in immune rats has been separated from the living cell, though not from living protoplasm.

AUTHOR'S SUMMARY.

GENETIC FACTORS IN THE ETIOLOGY OF MALIGNANT TUMORS. M. R. CURTIS, W. F. DUNNING and F. D. BULLOCK, Am. J. Cancer **17**:894, 1933.

Possibly when more is known about the etiology of other tumors (besides cysticercus sarcoma) for which there appears to be an inherited susceptibility, and when the expression of genetic factors in the cells and tissues is better understood, it will be found that in the case of all neoplasms, in all species, the initial cellular change occurs by a process analogous to somatic mutation, and that hereditary factors determine this change only so far as they influence longevity and the susceptibility of a person to some specific irritant or condition which is favorable to mutation.

AUTHORS' SUMMARY.

THE POTASSIUM CONTENT OF BENIGN UTERINE TUMORS. L. C. SCOTT, Am. J. Cancer **17**:924, 1933.

The means of potassium and ash were found to be 0.166 and 0.913 Gm. per hundred grams of moist tissue in ninety-eight benign uterine tumors, while the mean of moisture was 79.58 Gm. per hundred grams in ninety-seven tumors. The correlation coefficients were significant for potassium-ash and potassium-water, but not for water-ash. The mean, median and mode for potassium were the same for the ninety-eight tumors, taken collectively, and for forty-three tumors selected according to their macroscopic appearance and representing the usual type of tumor encountered. The potassium content of uterine fibromyomas is, in general, not in excess of that of normal tissue; in fact, the mean of ninety-eight tumors indicates that it is usually somewhat less.

AUTHOR'S SUMMARY.

QUANTITATIVE BEHAVIOR OF PROLAN A IN TERATOMA TESTIS. R. S. FERGUSON, Am. J. Cancer **18**:269, 1933.

A series of 117 consecutive cases of teratoma testis in which the quantitative excretion of prolan A has been studied is reported. The technic for the quantitative estimation of prolan A in the urine is described. The patient with teratoma testis will excrete from 50 to 50,000 mouse units of prolan A per liter of urine, depending on the embryonal character of the tumor, the extent of the disease and the status as regards treatment. Irradiation of the primary tumor and its metastases causes a decrease in the excretion of prolan A in the urine. Local recurrence or metastasis is accompanied by an increase in the excretion of prolan A in the urine, frequently before clinical detection of the lesion is possible. Serial observations at frequent intervals while the patient is under active treatment by irradiation give important prognostic information. Observations at autopsy reveal the important relation between the hormone of the anterior lobe of the pituitary and epithelial hyper-

plasia in the genital organs of the male, particularly the prostate and seminal vesicles. The significance of these observations is briefly discussed.

AUTHOR'S SUMMARY.

THE RELATION OF HEREDITY TO CANCER OCCURRENCE AS SHOWN IN STRAIN 73. MAUD SLYE, Am. J. Cancer **18**:535, 1933.

From consistent results obtained in this laboratory during twenty-three years and involving over 116,000 autopsies, the general trend indicates two facts clearly: 1. The genetic difference between susceptibility and immunity to cancer involves one gene; that is, they are unit characters. 2. In the stock in this laboratory susceptibility to cancer behaves like a recessive immunity, like a dominant.

AUTHOR'S SUMMARY.

THE INFLUENCE OF HORMONES ON THE GROWTH OF CARCINOMA, SARCOMA, AND MELANOMA IN ANIMALS. K. SUGIURA and S. R. BENEDICT, Am. J. Cancer **18**:583, 1933.

An investigation has been made of the effect of insulin, thyroxin U. S. P., parathyroid extract, epinephrine, theelin, theelol, emmenin, aqueous and alcoholic extracts of calf thymus, aqueous, alcoholic, ether and glycerin extracts of suprarenal cortex (sheep and cattle) and aqueous and alcoholic extracts of beef pituitary gland on Flexner-Jobling rat carcinoma, Jensen rat sarcoma, Seguira rat sarcoma, Gye mouse sarcoma 37, a transplantable mouse melanoma and Rous chicken sarcoma 1. The repeated subcutaneous or intramuscular injection of the hormones or the extracts of endocrine organs had no curative or retarding influence on the growth of transplanted tumors. Injection of aqueous extracts prepared from whole pituitary glands or from the anterior lobes had a slight but distinct stimulating effect on the growth of mouse melanoma and Seguira rat sarcoma. In the other tumors mentioned in this paper no stimulating action was observed. None of the other endocrine organ extracts or hormone preparations exerted any demonstrable accelerating influence on tumor growth.

AUTHORS' SUMMARY.

MULTIPLE MYELOMA. NORBERT ENZER and BENJAMIN LIEBERMAN, Ann. Int. Med. **8**:1062, 1935.

A case of multiple myeloma with features simulating hyperparathyroidism is reported. The possible rôle of the parathyroid glands in myelomatous and other generalized bone diseases is considered. In the case reported, atrophy of the parathyroid gland was encountered, and secondary or functional hyperparathyroidism is questioned. In both myelomatosis and hyperparathyroidism there is a disturbance of the calcium balance.

FROM THE AUTHORS' SUMMARY.

THE EFFECT OF MALIGNANT TUMOURS ON THE HYPOPHYSIS. C. S. MCEUEN, H. SELYE and D. L. THOMSON, Brit. J. Exper. Path. **15**:221, 1934.

In agreement with previous workers we have observed vacuolation and other histologic changes in the anterior lobe of the hypophysis in rats bearing transplantable tumors. Since, however, we have produced similar changes by intraperitoneal implantation of kidney tissue it is concluded that the changes produced by transplantable tumors are not necessarily related to the malignant growth as such but are at least partly due to decomposition of tissue in the necrotic centers of the tumors.

FROM THE AUTHORS' SUMMARY.

NUCLEUS-PLASMA RELATION OF CEREBRAL GLIOMAS. K. SCHAFFER, Beitr. z. path. Anat. u. z. allg. Path. **92**:199, 1933.

After a brief discussion of the classification of cerebral gliomas offered by Bailey and Cushing, Schaffer describes and pictures giant nuclei seen in spongio-

blastomas. Such nuclei indicate a nucleus-plasma relation altered in favor of the nucleus. This nucleus-plasma relation is evidence of extreme anaplasia or dedifferentiation and of neoplastic proliferation brought about by the abnormally active nucleus. Alteration of the nucleus-plasma relation in favor of the cytoplasm, the nucleus remaining passive, indicates a benign reaction of the glia to noxious agents of various kinds. In many glial tumors the nucleus-plasma relation may be altered in some cells in favor of the cytoplasm and in other cells in favor of the nucleus but without the formation of giant nuclei; cells with such a variable nucleus-plasma relation are termed dysgenetic. O. T. SCHULTZ.

VERTEBRAL CHORDOMA. O. SIMON, Deutsche Ztschr. f. Chir. **241**:805, 1933.

Vertebral chordoma is the rarest of the three types of chordoma, cranial, caudal and vertebral. Five cases of the vertebral type are reviewed from the literature, and one new case is reported. This occurred in a woman aged 57. The tumor grew from the anterior surfaces of the cervical vertebrae. The growth, which recurred after removal, was lobulated and divided by septums from the capsule. It was made up of small polygonal cells and larger cells with vaculoated plasma. Vertebral chordoma may arise from remains of the notochord within the vertebral body; it may grow intravertebrally or antevertebrally.

SKIN CARCINOMA IN A SCAR PROTECTED WITH INSULATING TAPE. W. BÜNGELER, München. med. Wchnschr. **81**:1619, 1934.

Within four months a squamous cell carcinoma developed in a scar (burn) on the back of the hand of a man aged 43 years. This rapid development is ascribed to the use of tape containing tar. By using benzine as a cleansing agent, the patient had satisfied the conditions for producing experimentally a tar carcinoma of the skin. AUTHOR'S SUMMARY.

A MESOGLIOMA OF THE BRAIN. W. K. BELEZKY, Virchows Arch. f. path. Anat. **290**:450, 1933.

In previously abstracted articles Belezky has presented the evidence that led him to conclude that the portion of the glia which is composed of oligodendroglia and Hortega cells is mesodermal in origin. This constituent of nervous tissue he terms "mesoglia" to distinguish it from the ectoglia of ectodermal origin. In this communication he describes the histology of a diffusely growing, infiltrative tumor of the left cerebral hemisphere of a youth aged 17 years. This tumor he believes to have arisen from the mesoglia; hence he terms it "a mesoglioma." He bases his opinion on the following characteristics, which indicate an origin from mesoglia: marked polymorphism of the cells; the presence of giant cells; the relationship of the tumor tissue to the numerous vessels of the tumor; the relation of the tumor cells to the walls of the vessels and the origin of those cells from the cells of the vessel wall; the presence of histiocytes, and the morphology and staining reactions of the tumor cells. In the classification of Bailey and Cushing the tumor would belong in the oligodendroglioma group, but Belezky disagrees with these and other authors that such tumors are of ectoglial origin. O. T. SCHULTZ.

INVOLVEMENT OF CRANIAL BONE IN MENINGIOMA. S. A. BERNSTEIN, Virchows Arch. f. path. Anat. **290**:500, 1933.

Bernstein presents the results of a microscopic study of the changes in cranial bone brought about by dural endothelioma or meningioma. The work was done in Erdheim's laboratory and is based on six tumors of the convex surface of the brain, five with and one without osseous involvement, and seven basal tumors, of which two were associated with involvement of bone. The malignant meningiomas consist of three parts, a subdural, a dural and an intra-osseous part. The

tumor invades the bone by the channels that carry the vessels, evoking a fibroplastic and osteoplastic reaction that precedes the invading tumor tissue. Tumors of the convex surface lead to thickening of the adjacent invaded bone by a laminated osteophytic layer, which may be present externally as well as internally. Tumor cells may disappear from the sclerotic and condensed bone. The blood vessels may be obliterated by the fibrotic process, resulting in necrosis of bone. Diffusely growing tumors may spread more widely and more rapidly in the bone than in the dura, and may penetrate the pericranium. The invading basal tumors caused sclerosis of bone but did not lead to thickening of the bones as did the tumors of the vault. In the basal tumors the invading tumor tissue itself formed bone. An appended note refers briefly to a benign meningioma that caused osteoporosis and absorption of the overlying bone.

O. T. SCHULTZ.

CRANIAL CHORDOMA. K. G. KLING, Acta path. et microbiol. Scandinav., supp. 16, 1933, p. 194.

A 45 year old woman died of pulmonary tuberculosis complicated by vague neurologic symptoms, particularly muscular atrophy. At autopsy there was found a tumor the size of a goose egg in the right posterior cranial fossa. It was made up of physaliphores (Virchow). These cells, which were in various stages of development, were arranged in islands and lobules. The tumor was limited by a capsule and had apparently grown gradually and slowly.

JACOB KLEIN.

MALIGNANT MIXED TUMOR OF THE UTERUS. RAGNAR OLINDER, Acta path. et microbiol. Scandinav., supp. 16, 1933, p. 314.

A 52 year old woman with menorrhagia was found to have a mixed tumor in the posterior wall of the corpus uteri. Microscopic examination showed the tumor to consist of a mixture of epithelial, sarcomatous and cartilaginous cells. Recurrence showed it to be malignant.

JACOB KLEIN.

PAPILLARY SYRINGOCYSTADENOMATOUS NEVUS AND ITS RELATION TO MALIGNANCY. O. REUTERWALL, Acta path. et microbiol. Scandinav., supp. 16, 1933, p. 376.

Four cases of this congenital type of cystadenomatous papillary tumor of the sweat glands are reported. The origin from sweat glands is determined only after microscopic examination. Malignant transformation of such tumors is of interest. There are groups of epithelial cells which have become separated during embryonic development and are often exposed to chronic irritation. In case 4 of this report biopsy showed an area of typical basal cell cancer with parakeratotic pearl formation. Another case is described from the literature.

JACOB KLEIN.

CONGENITAL PARTLY MALIGNANT NEOPLASM OF THE SCIATIC NERVE WITH CONSECUTIVE ELEPHANTIASIS. K. GROTH, Acta path. et microbiol. Scandinav. **11**:44, 1934.

In a new-born boy the entire right sciatic nerve from the origin to the ends of the branches was the site of a mixed tumor of mesodermal origin. The tumor contained cartilage and was in parts sarcomatous. In the intradural part of the nerve were three nodules consisting of convoluted nerve fibers and fibromatous tissue. The case shows that primary tumors may develop in the peripheral nerves from the connective tissue of the nerve without the involvement of the sheath of Schwann. Groth reviews sixty-six recorded cases of congenital sarcoma and finds no previous instance of primary congenital sarcoma in the peripheral nervous system. The cause of the elephantiasis, which was predominantly lipomatous, appears to have been a disturbance of the circulation of the extremity due to pressure on the common iliac vein by the tumor.

HETEROTYPICAL EPITHELIAL TUMORS OF THE SALIVARY GLANDS. AARNO SNELLMAN, Arb. a. d. path. Inst. d. Univ. Helsingfors **7**:419, 1933.

Eight cases of heterotypical tumors of the salivary glands are reported, with clinical and microscopic details. Measurements of nuclei and nucleoli tended to indicate that greater size of the nucleoli was associated with greater malignancy and cellular anaplasia. The microcentrum and the number of centrioles also reflected to some extent the degree of malignancy. The morphology and localization of the Golgi apparatus and the chondriosomes seemed in no way related to the degree of malignancy. The adenomatous structure, the cell function, the size of the nucleus and of the nucleoli and the morphology of the cells tend to place the growths in the same group as the mixed tumors.

JACOB KLEIN.

Medicolegal Pathology

FATAL ETHYLENE DICHLORID POISONING. W. C. HUEPER and C. SMITH, Am. J. M. Sc. **189**:778, 1935.

Ethylene dichloride, known since 1795 and first used as an anesthetic, has recently come into widespread commercial use as a solvent and cleaning fluid. A patient who drank 2 ounces (59 cc.) of this liquid died within twenty-two hours. Stupor, vomiting, diarrhea, cyanosis and subnormal temperature were the outstanding clinical symptoms. Death was apparently caused by circulatory failure. The ingested substance caused an extensive hemorrhagic colitis, nephrosis with marked tubular calcifications and generalized passive congestion of the internal organs besides multiple perivascular hemorrhages in the region of the cerebral basal ganglions.

FROM THE AUTHORS' SUMMARY.

TRAUMATIC RUPTURE OF THE HEART AND INTRAPERICARDIAL STRUCTURES. S. Z. HAWKES, Am. J. Surg. **27**:503, 1935.

This is a report of a study of seventy cases of injury to the heart and great vessels within the pericardium occurring in 2,708 deaths due to accidents. Such injuries may occur without fractures or visible evidence of external injury to the chest. In four cases the cardiac injury alone was responsible for death; in the other 66 cases there were associated ruptures of the lungs, liver, spleen or a hollow viscus or fracture of the skull or spine.

MAX LEDERER.

MEDICOLEGAL APPLICATIONS OF BLOOD-GROUPING, WITH SPECIAL REFERENCE TO THE AGGLUTINOGENS M AND N OF LANDSTEINER AND LEVINE. A. S. WIENER, Canad. M. A. J. **32**:393, 1935.

Blood grouping is of proved value in medicolegal cases of disputed paternity. Its application is based on experimental investigations of families involving tens of thousands of persons. Its reliability has also been established in various European countries in more than ten thousand medicolegal cases in which it has been applied. In this country the method has thus far been applied only in a small number of cases, and its introduction as a routine procedure in all cases of disputed filiation is urged. Blood grouping has also proved useful in criminal cases in which stains of blood, semen or saliva were found.

FROM THE AUTHOR'S SUMMARY.

RUPTURE OF THE DIAPHRAGM FOLLOWING SLIGHT TRAUMA. K. R. TATTERSALL and E. B. HARVEY, Brit. M. J. **1**:879, 1935.

A man was struck by a lorry. Four ribs were fractured; there were signs of left hemothorax and shortly after admission to the hospital signs of intra-abdominal disturbance. He died the next day. The autopsy showed a tear in the left side

of the diaphragm about 4 inches (10 cm.) long through which the kidney had been forced; the spleen was ruptured in four places, and there were fractures of anterior fossae at the base of the skull. With all these severe injuries there were no external marks of violence on the body.

MAX LEDERER.

RED COLORATION OF THE BODY IN BARBITURIC POISONING. F. DERVIEUX ET AL., Ann. de méd. lég. **14**:443, 1934.

Attention is called to the fact that in some cases of barbituric poisoning there may be a more or less widely spread rosy red coloration of the body after death.

THE CAUSE OF THE DEATHS ASSOCIATED WITH THE FOG IN THE VALLEY OF THE MEUSE. J. FIRKET, Ann. de méd. lég. **15**:2, 1935.

The illnesses and deaths attributed to the fog which collected over the valley of the Meuse in 1930 were associated with increased concentration of sulphurous anhydride in the atmosphere. In industrial cities greater concentrations of sulphurous anhydride are expelled into the atmosphere, but the gas is constantly driven away by numerous sources of heat, thus preventing the formation of a fog.

INTRAVASCULAR MIGRATION OF PROJECTILES. E. CURTILLET, J. de chir. **44**:715, 1934.

A case is described of a bullet wound in the pit of the stomach followed by migration of the bullet into the left femoral artery, from which it was removed successfully some weeks afterward. The cases of intravascular migration of projectiles recorded in the literature are reviewed and classified.

PRECIPITATING ANTIHEMOGLOBIN SERUM IN THE DIAGNOSIS OF BLOOD STAINS. B. BALOTTA, Boll. d. Soc. ital. di biol. sper. **9**:934, 1934.

Good results were obtained with antihemoglobin precipitin serum, human or animal, in the diagnosis of blood stains. The reactions were specific. No precipitates were formed when the antihemoglobin serum was mixed with sperm, bile or other protein-containing substances free from hemoglobin. Spots several years old gave good reactions. Similar results have been obtained with hemoglobin precipitins in identification of blood by Hektoen and Schulhof (*J. Infect. Dis.* **33**:224, 1923).

RELATION BETWEEN THE SUBGROUPS AND TRANSFUSION REACTIONS. N. BLINOV, Deutsche Ztschr. f. Chir. **243**:400, 1934.

Blinov studied 550 cases from the standpoint of the subgroups of blood group A and blood group AB. The ratio of subgroup A_1 to subgroup A_2 was found to be 4:1, and of A_1B to A_2B, 1.3:1. There were no fatal reactions when A_1 blood was given to persons with A_2 blood, but stronger reactions were obtained, on the whole, than when blood of the homologous subgroup was used.

A. S. WIENER.

TECHNIC OF DEMONSTRATING THE PROPERTIES M AND N. H. ELBEL, Deutsche Ztschr. f. d. ges. gerichtl. Med. **24**:242, 1935.

Elbel has adopted the method suggested by Lattes of absorbing the diluted immune rabbit serum with boiled erythrocytes when preparing specific testing fluids. This method, he thinks, has the advantage that it is possible to obtain colorless testing fluids. However, in my experience, it is possible to obtain practically colorless testing fluids even when fresh cells are used if the immune serums are properly inactivated before performing the absorption. Besides, if the testing fluid has a slight pink tinge, this in no way interferes with the tests, but rather

has the advantage that it serves to identify the testing fluid. And, as Elbel points out, boiled erythrocytes have lesser absorbing power than fresh blood, so that much more blood is needed, and the preparation of the testing fluids is more tedious since several absorptions are necessary.

A. S. WIENER.

GROUP SPECIFIC SUBSTANCES IN FRESH HUMAN MILK AND IN MILK STAINS. G. STRASSMANN and K. REISFELD, Deutsche Ztschr. f. d. ges. gerichtl. Med. **24**:330, 1935.

Tests for the presence of iso-agglutinins in fresh human milk yielded the following results: In twelve of nineteen specimens from group O persons both iso-agglutinins were demonstrable; in one only the anti-A agglutinin; in one only the anti-B agglutinin. In five neither iso-agglutinin was demonstrable. Tests for the group-specific substances in milk stains gave the following results: With none of seventeen stains from group O persons was nonspecific absorption observed, and in twelve of thirteen stains from group A and group B persons the corresponding group-specific substance was demonstrable. In the case in which the group-specific substance could not be demonstrated, the stain was taken from a "nonsecretor."

A. S. WIENER.

ERRORS IN TECHNIC IN FORENSIC BLOOD GROUPING TEST. A. LAUER, Med. Welt **8**:933, 1934.

The adoption by the courts of the tests for M and N in cases of disputed parentage doubled the chances of proving nonpaternity by blood tests. Many men who had not been excluded by tests previously made for the four classic Landsteiner blood groups applied for the newer M and N test in order to take advantage of the additional chance of exonerating themselves that these tests offered. Lauer thus had the opportunity of checking some of the results obtained by previous workers, and he found that in as many as eight cases errors in technic had been made despite the relative simplicity of the ordinary blood grouping tests. In not one of the cases in which errors had been made was the person who made the tests a serologist. Furthermore, the tests had been made with commercial serum, adequate controls had not been used, and the results had not been properly checked by testing each unknown serum against known cells. Errors of this sort can serve only to shake the confidence of the courts in these valuable tests. Of course, the difficulty is not with the tests but with the persons who make the tests. It is therefore essential, especially with the more difficult tests for M and N, to permit only properly qualified persons to perform the tests in medicolegal cases.

A. S. WIENER.

EMBOLIC TRANSMISSION OF A RUBBER CATHETER INTO THE HEART IN ABORTION. J. BLÁHA, Zentralbl. f. Gynäk. **59**:746, 1935.

A woman requested a medical examination because on the previous day she had attempted an abortion by means of a rubber catheter. There was a perforation of the cervix. Laparotomy was resorted to, but a catheter could not be found. Curettage disclosed that there had been an incomplete abortion, and it was assumed that the catheter had induced the abortion and had been expelled without the woman noticing it. Sepsis developed and the woman died on the seventh day. At necropsy the lower, rough end (evidently broken off) of the catheter, which was 23.5 cm. long and 4 mm. thick, was detected in the inferior vena cava near the diaphragm. From there the catheter extended through the right auricle, the superior vena cava and the innominate vein into the left common jugular vein, in which the upper, smooth end of the catheter was found several centimeters above the clavicle. A comparison of the conditions at necropsy with the clinical course indicates that, following perforation of the cervix, the catheter must have entered a wide vein of the uterine plexus, from which it slipped directly into the right

hypogastric vein and into the inferior vena cava. It may be deduced from a number of symptoms that the catheter reached the heart on the fifth day following its introduction into the uterus. At any rate the catheter must have been in the heart for about forty-eight hours without causing severe impairment of the heart action. The author reviewed the literature on foreign bodies in the heart and found that in the majority of cases the foreign body was a projectile. He thinks that the reported case is the first one in which such a large foreign body entered the heart by embolic transmission. He reviews cases from the literature in which a catheter disappeared following introduction into the uterus. In some cases the catheter was extracted from the uterus; in others it entered the retroperitoneal tissue and was later extracted from an abscess, in others it entered the preperitoneal tissue and caused an abscess in the anterior abdominal wall; in others it entered Douglas' pouch, and in a considerable number of cases it entered the free abdominal cavity and produced diffuse peritonitis.

EXAMINATION FOR ALCOHOL IN PERSONS INJURED IN ACCIDENTS. J. HINDMARSH and P. LINDE, Acta chir. Scandinav. **75**:198, 1934.

At a hospital in Stockholm persons injured by accidents and admitted to the surgical clinic in the course of twelve months were subjected to a special examination with a view to finding out whether there had been any consumption of alcohol. The clinical examination was at times difficult on account of the patients' injuries. It was supplemented, however, by determination of the alcohol in the blood by Widmark's micromethod. It seems to the authors that this method is well suited for clinical use. It is emphasized, however, that difficulties may be encountered in making the test with patients affected by shock. In the present material good agreement is shown to exist between the clinical diagnosis and the alcohol content of the blood. The influence of alcohol even in low concentrations has generally been recognized. Widmark's earlier data on this point are discussed. It is emphasized that in cases of severe injuries one must often refrain from forming a judgment of the victim's state of sobriety. Accidents under the influence of alcohol are of common occurrence in traffic. It has been shown that also in the case of accidents during work a considerable amount of alcohol has sometimes been consumed. On admission to the hospital 41 per cent of all males injured in accidents (283) had alcohol in the blood, and of these as many as two thirds had more than 1 part per thousand. Those injured in traffic (115) showed about the same rate. Accidents under the influence of alcohol are commonest among more elderly people. Furthermore accidents tend to accumulate over week-ends and in relation to bank holidays. It is stated as a noteworthy fact that more than half of those who have overturned in driving a car or a horse, or who have fallen out of vehicles, or who as pedestrians have been knocked down, have had alcohol in the blood. This supports the view that in traffic accidents it is not only the driver of the vehicle who should have his state of soberness tested but also the injured persons.

FROM THE AUTHORS' SUMMARY.

RESULTS OBTAINED FROM MEDICOLEGAL APPLICATION OF THE MN AND FOUR GENE THEORIES. E. WOLFF and B. JONSSON, Acta path. et microbiol. Scandinav. **12**:131, 1935.

This excellent analysis of the theories as to the heredity of the blood groups and the MN types is based on data accumulated from a study of 600 cases of disputed paternity. The blood specimens were sent from all parts of Sweden, and it was shown statistically that the material was homogeneous from the standpoint of the distribution of the agglutinogens. Among the 1,200 adults tested, 37.92 per cent were of group O, 36.92 per cent of subgroup A_1, 9.75 per cent of subgroup A_2, 10.33 per cent of group B, 3.92 per cent of subgroup A_1B and 1.17 per cent of subgroup A_2B. Furthermore, 36.08 per cent belonged to type M, 47 per cent to type MN, and 16.92 per cent to type N. Hence, $p + q + r = 1.00144 \pm 0.00400$,

and does not differ significantly from unity. This test combined with a special analysis of the data in groups of 50 persons each supports the Bernstein theory of heredity of the blood groups. Furthermore, $m + n = 1.01200 \pm 0.01443$, so that the data also support the theory of Landsteiner and Levine as to the heredity of the agglutinogens M and N. Additional special statistical tests add further support to these theories, and also Thomsen's theory of the heredity of subgroups of group A and group AB. With special regard to the agglutinogens M and N, 709 mothers with 732 children were examined, but not a single exception to the theory of Landsteiner and Levine was found. In all, 5,835 mothers with 7,759 children have been studied by various investigators thus far. The authors therefore believe that exclusions of paternity based on the MN theory should be considered on the same level as those based on Bernstein's theory. More caution is required when applying Thomsen's theory, since the subgroups have not been as thoroughly studied yet.

A. S. WIENER.

INCOMPATIBILITY OF THE BLOOD OF THE SAME GROUP DEPENDING ON AGGLUTININ FOR HITHERTO UNKNOWN RECEPTORS. A. ZACHO, Hospitalstid. **70**:225, 1935.

On account of severe hemorrhage on the separation of the placenta, the patient was given transfusions of blood from three donors of the same blood type as herself. During the subsequent days there were signs of disintegration of red blood corpuscles. Six days later another transfusion of 100 cc. was given. The patient died five hours later. The donors and the patient were all of type A_1, but direct cross-matching tests of the cells and serums of donors and recipient were not made. This was done later and it was found that the patient's serum agglutinated the red corpuscles of two of the donors. The agglutinin in question appears not to have been recognized before; it agglutinated the red corpuscles in about 60 per cent of persons with O and A types of blood.

Technical

PARAFFIN SECTION METHOD FOR THE DOPA REACTION. S. W. BECKER, L. L. PRAVER and H. THATCHER, Arch. Dermat. & Syph. **31**:190, 1935.

The dopa reaction was performed en bloc by the simplified technic of Laidlow and Blackberg. The tissue was then fixed in Bouin's solution, embedded in paraffin and cut into thin or into thick sections if such are desired for a study of dendritic melanoblasts in their entirety. The sections may be stained by many methods, the most valuable of which for cytologic study is the Masson trichrome (iron hematoxylin, acid fuchsinponceau de xylidine and aniline blue), for which very thin sections must be used. All the sections showed that the dopa reaction does not occur in palisade basal cells, and no transitional forms were found between the palisade basal cells and the melanoblasts. These observations support the conception of the presence of two types of cells in the epidermis, one of which has to do with the regulation of pigment function. This method should make possible a more intelligent study of pigmentary processes by the dopa reaction than has been possible by the frozen section method. FROM THE AUTHORS' SUMMARY.

A NEW METHOD OF PRESERVING NORMAL AND PATHOLOGIC BRAIN TISSUE. JOSHUA ROSETT, Arch. Neurol. & Psychiat. **32**:513, 1934.

For the preservation of brain specimens for teaching or other purposes Rosett recommends a new method which is devoid of the objectionable features in the use of fluids such as formaldehyde or alcohol. The new method consists in infiltrating the tissues with the insoluble, infusible synthetic resinoids of the phenol series. Slabs of brain tissue of any thickness preserved in formaldehyde are first placed for about one week in a 40 per cent solution of formaldehyde and then transferred to the foregoing solution of resinoids for ten days or longer, depending on the

thickness of the slabs. After this they are subjected to polymerization, ground, polished and in some instances developed (in a solution of sodium hydroxide and salicylic acid). Certain precautions must be taken in handling the solution of the resinoids, for the description of which the original article should be consulted.

GEORGE B. HASSIN.

CEREBROSPINAL FLUID IN TUBERCULOUS MENINGITIS. H. HOUSTON MERRITT and FRANK FREMONT-SMITH, Arch. Neurol. & Psychiat. **33**:516, 1935.

From a study of 297 cerebrospinal fluids in 84 verified cases of tuberculous meningitis it was found that aside from an increase in the pressure and in the number of white cells (lymphocytes predominating) there was a decrease in sugar content below 45 mg. per hundred cubic centimeters and of chlorides below 650 mg. per hundred cubic centimeters. In the early stages the results may not be typical. The pressure and the cell count, for instance, may be normal, but the contents of sugar, chlorides and protein are usually abnormal. No difficulties are encountered in differentiating the condition from pneumococcic, staphylococcic, streptococcic and other types of meningitis, but it may be difficult to differentiate the condition from syphilitic meningitis, especially in the absence of typical serologic reaction.

G. B. HASSIN.

A SIMPLE METHOD FOR STAINING THE NERVE TISSUES. E. H. LOUGHLIN, Arch. Neurol. & Psychiat. **33**:616, 1935.

Loughlin describes a combined gold and silver staining method which can be used not only on fresh brain tissues but also on tissues that have been fixed in solutions of formaldehyde for years, or in other solutions (Zenker's fluid, for instance), or that have not been fixed until several days after necropsy. Loughlin's modification contains the essential features of the methods of Cajal and Bielschowsky. Frozen sections are preferable, from 15 to 18 microns thick, but paraffin and pyroxylin (celloidin) sections also give good results. As a mordanting agent, Loughlin uses dilute (2 per cent) bromine water (instead of hydrobromic acid) to which the frozen sections are transferred after they have been rinsed in water and kept in 5 per cent ammonia over night. For the details the original article should be consulted. As the photomicrographs show, the method brings out clearly the ganglion and various glia cells, mature and embryonic, their processes and the fat granule bodies, but less clearly the microglia.

G. B. HASSIN.

THE LABORATORY DIAGNOSIS OF AMEBIASIS. T. B. MAGATH, J. A. M. A. **103**: 1061, 1934.

The direct smear method is adequate in the hands of those properly trained in almost all cases but, if doubt exists, one should resort to fixed and stained preparations. Whether one uses formed stools or those resulting from catharsis will depend on the individual problem. Whichever is used, the limitations of the particular method must be clearly recognized.

Culture methods should be used in laboratories qualified to identify amebas, but, for the usual routine, cultures are not necessary, provided the examiner thoroughly knows how to make proper direct examinations. The cultural characteristics of various amebas growing in these cultures have yet to be clearly described, and a series of animal experiments to determine the types in a large series is greatly needed.

Until the complement-fixation method is simplified, it is not suitable for routine tests.

Seventy-five per cent of infestations with Endamoeba histolytica can be found by examining a single stool following catharsis with magnesium sulphate, whereas only a third of the infestations will be found by examining a single formed stool. It will require from eight to ten formed stools to establish the same number of infestations as three stools following catharsis.

FROM AUTHOR'S SUMMARY.

Society Transactions

AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

Twenty-Second Annual Meeting, Hotel Statler, Detroit, April 10-13, 1935

S. Burt Wolbach, *President*

Shields Warren, *Secretary*

The Bone Marrow in Patients with Cirrhosis of the Liver. Raphael Isaacs, University of Michigan.

In patients with cirrhosis of the liver, the number of red blood cells larger than normal is increased. In some patients the blood picture simulates that of pernicious anemia. The bone marrow of 6 patients having cirrhosis of the liver and anemia was studied with reference to the number of cells per cubic millimeter and its differential composition of the types of nucleated cells. It was found that the number of red blood cells in the megaloblast stage is significantly increased, correlating with (*a*) the macrocytosis of the mature red blood cells and (*b*) the favorable therapeutic response to liver extract.

Effect of Pressor Reactions on Bacterial Localization in the Central Nervous System. William F. Petersen and A. J. Nedzel (by invitation) University of Illinois.

At the last meeting of the society we presented evidence indicating that pressor episodes were of significance for the localization of bacteria on the cardiac valves and demonstrated in dogs the experimental production of endocarditis when staphylococci and streptococci were injected intravenously shortly after the maximum pressor effect from injections of pitressin.

We have extended our observations to the localization of bacteria in the central nervous system. We used single as well as repeated injections of pitressin and a solution of the diuretic-antidiuretic hormone of the hypophysis (betahypophamine) to bring about transient anoxemia of the central nervous system and therewith foci of anoxemic stimulation where localization of bacteria might be enhanced.

In the control animals we depended wholly on the possible passage of the normal flora of the mucous membranes; in the experimental animals the mucous membrane of the throat was sprayed with cultures of Staphylococcus and Bacillus megatherium.

In the case of a single injection of pitressin the numbers of bacteria seen in the region of the hippocampus major and in the spinal cord were small, similar to the findings in the normal dog without any injection of pitressin. In dogs that received a series of injections we found considerably larger numbers of microorganisms, the bacteria often forming groups; there was also distinct evidence of pathologic change in the nervous tissue. These changes are now being studied.

The findings mentioned lead us to the conclusion that pressor effects, no matter how induced, may lead to the localization of bacteria and to tissue change in places where these pressor effects (vascular spasm) are particularly pronounced.

Pertussis: Production of an Interstitial Mononuclear Pneumonia with the Bordet-Gengou Bacillus. Douglas H. Sprunt and (by invitation) Donald S. Martin and Jarrett E. Williams, Duke University School of Medicine.

The presence of an interstitial mononuclear pneumonia in a patient with pertussis led McCordock to suggest that a virus may be the primary etiologic agent in this

disease. This point of view was based on his preceding work with Muckenfuss on the relationship between viruses and this type of pneumonia. Rich has further substantiated this relationship.

In recent experiments we showed that exotoxins are capable of producing interstitial mononuclear pneumonia when injected intratracheally into rabbits. This work led us to try similar experiments with the Bordet-Gengou bacillus.

Pure cultures of this bacillus were kept in phase I by being grown on the Bordet-Gengou medium containing 25 per cent human blood. Twenty rabbits were inoculated intratracheally with a suspension of the cultures. All the rabbits acquired a typical interstitial mononuclear pneumonia. As a control, 5 rabbits were given injections of cultures of the rough form of the Bordet-Gengou bacillus, grown on a similar medium, without producing any changes in the lung.

Before the experiment the nares of the rabbits were swabbed and the material cultured for Bacterium lepisepticum and Bacterium bronchisepticum, with negative results. At necropsy material from the lungs was cultured for organisms other than the Bordet-Gengou bacillus, without success.

Conclusion.—The Bordet-Gengou bacillus can produce interstitial mononuclear pneumonia in rabbits.

SUSCEPTIBILITY TO TOXIC AGENTS ALTERED BY VARIATIONS IN THE GLYCOGEN AND FAT CONTENT OF THE LIVER. JESSE L. BOLLMAN and FRANK C. MANN, The Mayo Clinic.

The composition of the liver changes throughout the day in relation to the amount and character of the food taken. The effects of toxic agents such as alcohol, chloroform, carbon tetrachloride or tetrachlorethane appear more marked if these agents are administered when the glycogen content of the liver is low. This may be noted by the systemic effects of the drugs and by the extent of the pathologic changes produced in the liver. Diets rich in carbohydrates raise the amount of glycogen in the liver as much as five times that found with the usual mixed diet. Diets containing excessive fat may alter the fat content of the liver to ten times the usual one. Susceptibility to toxic agents is increased with fat feeding and decreased with carbohydrate feeding. Attention is also called to the rapid changes in the composition and histologic picture of the liver produced by diet or by toxic agents.

STUDY OF STANDARD BREEDS OF RABBITS IN RELATION TO A TRANSPLANTABLE MALIGNANT TUMOR. ALBERT E. CASEY, The Rockefeller Institute for Medical Research and the University of Virginia.

In the laboratory of Dr. Wade H. Brown at the Rockefeller Institute for Medical Research sample rabbits from 15 standard breeds were propagated in pure line over a period of from three to ten years. None of the breeding stocks were used for experimental purposes during this period, and they were kept in the same rooms under identical conditions of food, light, air, caging and attention. More than 250 healthy young adult males of approximately the same age from the families representing the 15 standard breeds (English, American Blue, Beveren, Flemish, Havana, Rex, French Silver, Polish, Himalayan, Belgian, Dutch, Sable, Special Albino, Tan and New Zealand) were inoculated in large groups with the same dose and emulsion of the Brown-Pearce rabbit tumor. This tumor had been carried in this laboratory for ten years through mongrel stocks purchased from outside dealers and consisting largely of Belgian, Flemish, Dutch and Beveren mixtures. The tumor, therefore, was not adapted to the standard breeds used for the test. Two months after the inoculations (intratesticular) the surviving animals were killed and complete autopsies done. The amount of primary and of metastatic tumor was measured by water displacement, and the number of metastatic foci per animal computed. It was found that the breeds varied from almost 100 per cent susceptibility on the part of the English, American Blue and Sable types to almost 100 per cent resistance to the tumor on the part of the Havana and Himalayan breeds. Analyzed statistically, the variance between the breeds was significantly

greater than the variance within the breeds ($P = 0.01$—). This difference was of statistical significance as regards the incidence of primary tumors, the incidence of metastases, the number of metastatic foci per animal, the total volume of metastatic tumor per animal, the actual mortality, the total mortality and the longevity after inoculation. These seven criteria were found to be reliable indexes of various phases of malignant growth in earlier experiments with this tumor. Of the 25 English animals used, 92 per cent acquired primary tumors, and 100 per cent of those in which primary tumors developed acquired metastases. The total volume of metastatic tumor per animal was 94 cc., and the number of metastatic foci per animal was 17.5. The actual mortality was 76 per cent, the expected mortality 84 per cent, and the total longevity forty-four days. This compared with an almost 100 per cent resistance on the part of the Havana breed. Of the 18 animals in this breed 22 per cent acquired primary tumors. Of the 4 in which primary tumors developed, 50 per cent (2) had metastases; the volume of metastatic tumor per animal was 11 cc., and the number of metastatic foci per animal was 3. The actual mortality was 0 per cent, the expected mortality 11 per cent, and the total longevity sixty days (all animals were living at the termination of the experiment). The English and the Havana breeds were at opposite extremes on almost every point of malignancy analyzed. The differences between the 2 rabbit breeds with the transplantable tumor are in every respect comparable with the results found in mice and rats with spontaneous tumors by selective inbreeding. This is, perhaps, the first study of numerous metastatic phases of malignancy in genetically different breeds of a mammal. Since this tumor metastasizes in various portions of the body in every way comparable to human tumors, and actually kills the animal by metastatic involvement of vital organs, the results are perhaps more applicable to the situation found in connection with human tumors than are the results of previous genetic studies of spontaneous tumors or of studies on transplanted tumors of small rodents with very low metastatic rates. Furthermore, the degrees of susceptibility to the tumor shown by the 15 breeds coincide fairly closely with their degrees of susceptibility to experimental syphilis, spontaneous rabbit pox and spontaneous pneumonia, and with hereditary variations in their size, blood formulas and breeding characteristics, with certain individual exceptions.

ARE THERE LIMITS TO THE CAPACITY FOR IMMUNOLOGIC RESPONSES? REUBEN L. KAHN and (by invitation) ELIZABETH B. MCDERMOTT, University of Michigan.

This is a report of progress in studies in which it is aimed to determine the extent to which increasing the number of immunizing injections of a protein antigen in an animal proportionately increases the immunologic capacities of the animal. The antitoxin-toxin method of measuring the capacity of the tissues of an immunized animal to combine with specific antigen in vivo was employed for the determination of the immunologic response. Five groups of rabbits were immunized with horse serum by means of, respectively, one, two, three, four and five injections of 0.2 cc. per kilogram of body weight, given at ten day intervals. Each immunized rabbit was given 50 M.L.D. of diphtheria toxin and simultaneously a designated amount of horse serum antitoxin for diphtheria, considered as specific antigen, intracutaneously, about 5 cm. distant. Differences in the capacity of the skin to "anchor" the antitoxin, thereby preventing its diffusion from the area of injection and its neutralization of the toxin, were indicated by differences in the number of units necessary to save the animals from death due to toxin. Thus, control rabbits not immunized with horse serum required 20 units of antitoxin for protection against 50 M.L.D. of toxin. Rabbits that had received one immunizing injection of horse serum required 300 units of antitoxin and were not protected by 200 units. Rabbits that had received two immunizing injections were protected by 1,000 units but not by 750 units. Rabbits that had received three immunizing injections were protected by 2,000 units but not by 1,750 units. As a result of four and five immunizing injections, respectively, 2,500 units was required to protect, 2,000 units having failed to protect. Increasing the number of immunizing injections of horse serum

evidently did not result in a proportional increase in the capacity of the skin to combine with the horse serum antitoxin. Similar results were obtained with a series of rabbits that were given six immunizing injections. It was also observed that the increase in the number of injections did not result in a proportional increase in serum precipitins. These studies suggest that the capacity for immunologic response is manifested to a moderate degree as a result of a first immunizing injection of protein antigen; that this capacity rapidly increases after a second and a third injection, respectively, and that the increase is comparatively small after a fourth, and still smaller after subsequent injections, indicating, in turn, that there are limits to the capacity for immunologic responses.

EFFECTS OF LOCAL IMMUNIZATION OF THE UPPER RESPIRATORY TRACT OF RABBITS AGAINST PNEUMOCOCCUS, TYPE I. THEODORE E. WALSH (by invitation) and PAUL R. CANNON, The University of Chicago.

Rabbits were immunized intranasally with a formaldehydized vaccine of Pneumococcus, type I, and their resistance to intranasal instillation of living virulent pneumococci determined. Control animals were treated intranasally with a vaccine of paratyphoid bacilli, with 0.4 per cent solution of formaldehyde or with 1 per cent solution of tannic acid. The object was to ascertain to what extent local immunization of an important portal of entry may protect a susceptible animal against infection by the same route, and to determine whether such resistance is predominantly local or general, specific or nonspecific. The results may be summarized as follows:

Eighteen of 20 normal animals infected intranasally with a saline suspension of living cultures of Pneumococcus, type I, died of a pneumococcic septicemia within from two to three days.

None of the 17 rabbits which had been immunized intranasally for from five to nine days with the formaldehydized pneumococcus vaccine died after intranasal infection with living cultures of this organism.

Eleven of 12 rabbits treated by intranasal instillation of a vaccine of paratyphoid bacilli, 0.4 per cent solution of formaldehyde or 1 per cent solution of tannic acid succumbed to the intranasal application of living cultures of Pneumococcus, type I.

We conclude from these experiments that a susceptible animal can be immunized intranasally by a specific bacterial vaccine so that subsequent intranasal infection leads to no serious invasion of the blood stream. Nonspecific stimulation of the tissues of the upper respiratory tract apparently confers no protection under similar conditions. Further experiments are in progress in an attempt to determine to what extent the increased resistance depends on local or on general processes of immunity.

FURTHER OBSERVATIONS ON THE CELLULAR CONSTITUENTS OF BOVINE SYNOVIAL FLUID. GRANVILLE A. BENNETT and (by invitation) CHARLES F. WARREN and WALTER BAUER, Harvard University Medical School and Massachusetts General Hospital.

Under varying conditions, cytologic examinations have been made on specimens of synovial fluid from the astragalotibial and carpometacarpal joints of cattle and from the knee joints of dogs. From the data tabulated it has been possible to correct an error made in this laboratory concerning the classification of certain nucleated cells. The results may be summarized as follows:

When one compares specimens of synovial fluid from the astragalotibial and carpometacarpal joints of young beef cattle, one observes wide variations in the total numbers of nucleated cells and in the percentages of the individual cell types. The greatest variation in the percentages of the cell types is noted in astragalotibial joint fluid.

The total numbers of nucleated cells and the percentages of phagocytic cells are greater in the carpometacarpal joint fluid than in the fluid from the astragalotibial

joint. This difference is best explained by the increased amount of débris in the carpometacarpal joint resulting from the presence of defects in the articular cartilage.

The wide variations in the numbers of the individual cell types observed in the astragalotibial joint fluid are evidently within the limits of the so-called normal. The normal figure depends on the degree of wear and tear and minor traumas to which the joint has recently been subjected. Evidently irritations of this grade are sufficient to increase the total number of nucleated cells and the percentage of phagocytic cells exclusive of polymorphonuclear leukocytes.

The total number of nucleated cells contained in synovial fluid may increase post mortem, but there is very little change in the percentages of the individual cell types.

Reexaminations of specimens of synovial fluid from two and one-half to three hours after the first count was made invariably revealed a marked increase in the number of dead or degenerating cells, together with considerable clumping. Although the relative percentages of phagocytic cells were not materially altered, it became increasingly difficult to recognize distinguishing characteristics of the phagocytic cells.

Several methods of study failed to reveal any evidence to support a theory that the cytology of synovial fluid is a reflection of that of the blood.

A definite species difference in the total numbers of nucleated cells and the percentages of individual cell types contained in normal synovial fluid has been pointed out.

The tabulated results obtained from this study should enable one to interpret more accurately the cytologic variations in normal and pathologic human synovial fluid.

CHANGES IN THE PITUITARY GLAND FOLLOWING THYROIDECTOMY IN RATS. ISOLDE T. ZECKWER, University of Pennsylvania.

The pituitary glands of white rats thyroidectomized when immature showed at fourteen days following operation an increase in the number of basophils and a decrease in that of acidophils; at thirty days, an almost complete disappearance of acidophils and an accumulation within basophils of large globules of smooth hyaline material, and as time increased up to two hundred and ninety days, condensation of the hyaline material into single globules within basophils, which caused these to simulate "castration cells" and which looked like increased production and storage of secretion. It is suggested that the dwarfing of cretins is due to loss of acidophils. Fat stains revealed no fatty degeneration. Acidophils appeared to disappear by loss of granules and dedifferentiation rather than by degeneration. Implantation of pituitary glands into guinea-pigs revealed an abundance of thyrotropic hormone in the pituitary glands of cretins, and it is likely that the amount of hormone available per gram of body weight of the cretin dwarf is greater than that of controls. Thyrotropic hormone cannot have its source in acidophils. It is suggested that basophils may produce thyrotropic hormone. Male cretins showed large adrenal glands and testes in relation to body weight. This suggests that there may be alterations in the proportions of adrenotropic and gonadotropic hormones coincident with the altered proportions of histologic types of cells.

PATHOLOGIC CHANGES RESULTING FROM ACCURATELY CONTROLLED ARTIFICIAL FEVER. F. W. HARTMAN and (by invitation) R. C. MAJOR, Henry Ford Hospital.

The character of the lesions produced in the adrenal glands and brain were studied in detail as a basis for explaining the vascular collapse and cerebral symptoms that are noted following administration of artificial fever both in man and in experimental animals.

EFFECT OF ARTIFICIAL FEVER ON ACUTE TUBERCULOSIS IN EXPERIMENTAL ANIMALS. R. C. MAJOR and H. P. DOUB (introduced by F. W. HARTMAN, Henry Ford Hospital.

Acute tuberculosis has been produced in dogs and monkeys, and the technic particularly of producing tuberculosis in the lung is described. The progress of the pulmonary lesion has been followed with roentgenograms, temperature charts and smears for tubercle bacilli. The animals were exposed to artificial fever in the Simpson-Kettering cabinet for periods of from four to five hours at a temperature of from 104 to 107 F. at weekly intervals. This type of artificial fever does not seem to be harmful, and many of the lesions are affected favorably, as indicated by a disappearance of bacilli from the sputum, a return to normal temperature and a disappearance of the roentgen evidence of lesions.

DETERMINATION OF BODY SURFACE AREA BY PHOTOGRAPHY. JOHN C. BUGHER and DAVID H. DRUMMOND (introduced by CARL V. WELLER), University of Michigan.

Two methods are presented: In the first, the surface area is determined by mechanical quadrature, the body being assumed to be divided into approximately 100 cross-sections of uniform thickness. The perimeters of these elements are computed by considering the cross-sections as ellipses whose major and minor axes are the corresponding dimensions of the actual sections. The skin surface of each section is obtained by multiplying the perimeter by the average slant height. The summation of these elemental areas gives the total body area. The diameters are obtained from frontal and lateral photographic silhouettes. The probable error is approximately 2.5 per cent.

In the second method, which is a further development of the first, the measurements necessary are: (1) the areas of the two silhouettes by planimeter, (2) the perimeters of the silhouettes by chartometer and (3) the height. By means of constants, the surface area may be quickly calculated. This method is slightly less accurate than the first but is more easily employed.

Both methods are independent of variations in body contour, so that the deviations encountered in the application of height-weight formulas do not occur.

THERAPEUTIC AND PROPHYLACTIC LIMITATIONS OF POLIOMYELITIS IMMUNE SERUM. E. W. SCHULTZ and (by invitation) L. P. GEBHARDT, Stanford University.

Twenty-four experiments were carried out in an effort to determine the therapeutic and prophylactic value of poliomyelitis immune serum. An immune horse serum of high virucidal titer was employed. This was administered by different routes and for the most part in large doses. Thirty-six monkeys received serum one or more days after inoculation with virus, and 91 monkeys received serum one or more days before inoculation. No therapeutic effect could be demonstrated in the former group, and less than a fourth of the animals in the latter group escaped the disease. Well defined protection was obtained only against small doses of virus.

The results are explained on the basis of recent observations which indicate that the virus is not only a highly neurotropic but probably an intracellular parasite. Once it becomes established in the olfactory nerve, it no longer can be reached by immune serum. Used prophylactically, serum has the effect of diminishing the amount of virus able to initiate infection. This is evidenced by a longer average incubation period in the serum-treated group. However, a dose of virus just sufficient to infect eventually leads to as extensive paralysis as is found in controls.

EXPERIMENTAL STUDIES DEMONSTRATING A RECIPROCAL RELATIONSHIP EXISTING BETWEEN LYMPHOPOIESIS AND MYELOPOIESIS. B. K. WISEMAN, C. A. DOAN and (by invitation) L. A. ERF, Ohio State University.

The introduction of simple proteins and of nucleic acid derivatives into the blood streams of two separate groups of rabbits resulted in characteristic changes

in the structures of the lymphatic and myeloid tissues. These alterations were reflected in a disturbance of the proportionate distribution of the cells in the circulating blood. Those animals receiving native protein (egg albumin, horse serum, etc.) showed a specific rise in the absolute numbers of lymphocytes accompanied by a striking decrease in the numbers of circulating neutrophilic leukocytes. Examination of the lymphatic and myeloid tissues during the period in which this disturbed relationship was prominent revealed hyperplastic changes in the former, associated with a striking hypoplasia in the latter.

In contrast to this reaction, the animals receiving nucleoprotein derivatives (nucleic acid, nucleotides) exhibited a marked neutrophilic leukocytosis with absolute lymphopenia. The tissue changes consisted of increased myelopoiesis (hyperplastic bone marrow and ectopic foci of granulopoiesis) associated with widespread degeneration of lymph structure (atrophy of lymphoid follicles and cyst formation).

Similar reciprocal changes in the tissues and blood of clinical patients showing abnormal lymphoid or myeloid stimulation have been observed and studied, and a correlation made with the changes observed in the experimental animals. These reciprocal hypoplastic and hyperplastic changes in the myeloid and lymphatic tissues tend to establish further the existence of a close relationship between the respective functional capacities of the hemopoietic organs, and they have important practical and theoretical implications that are of basic importance in the interpretation of the physiology and pathology of blood formation.

A Study of Bile from the Human Gallbladder. Cecilia Riegel, I. S. Ravdin and P. J. Morrison, University of Pennsylvania.

Bile Obtained from Patients with Noncalculus Cholecystitis.—The bile removed at operation from patients whose condition was diagnosed as noncalculus disease of the gallbladder has been studied, and the results have been compared with those obtained on bile from the normal human gallbladder. The cases were separated into two groups—visualized and nonvisualized—according to whether the gallbladder was or was not visualized after the administration of sodium tetraiodophenolphthalein.

In both the visualized and the nonvisualized group the concentrations of chloride and bile salt were different from normal, the chloride being higher, the bile salt lower. No marked difference was found between the two groups in any of the various normal constituents studied. There was a tendency for the cholesterol concentrations to be somewhat higher in the nonvisualized group, but this was by no means constant. It was also true that the lowest calcium concentrations were found in the nonvisualized group.

The data are interesting in that they may show some of the initial changes which precede cholelithiasis.

Bile from Patients with Calculus Cholecystitis.—We have carried out chemical analyses of the bile or stones or both taken from the gallbladder at the time of operation in 75 cases of calculus disease of the gallbladder. Cholecystographic reports were available in many of these cases. The cases have been classified into two groups—visualized and nonvisualized—according to whether the shadow of the gallbladder was or was not observed. The groups were further subdivided as to whether the stones were visualized as a positive or as a negative shadow. The data show a lack of correlation between visualization of the stones and chemical composition of the stones; the calcium content of the visualized stones was no higher than that of the nonvisualized ones. With two exceptions the stones contained chiefly cholesterol with varying amounts of calcium, pigment, phosphate, carbonate, organic débris, etc.

Analysis of the bile taken from the gallbladder at operation brought out several interesting points: In all cases the chemical composition of the bile was markedly altered from the normal. The chloride content was higher, the bile salt lower. In the visualized group the chloride content was as a rule lower, and the bile salt and cholesterol content higher, than in the nonvisualized group. In both groups the bile salt-cholesterol quotient tended to be lower than normal.

These experiments, taken in conjunction with previous work on normal and damaged gallbladders of dogs, on the composition of bile from human gallbladders with noncalculus cholecystitis, and on that of human hepatic bile after damage to the liver, indicate that in the final production of gallstones many varied factors may be involved.

Bile from Pregnant Women at Term.—Thirty-five specimens of bile obtained from pregnant women were analyzed for their concentrations of calcium, chloride, bile salt, cholesterol and phosphate. The data are significant in that they show in the majority of instances consistent change in the ability of the gallbladder membrane to concentrate bile. The concentrations of chloride, calcium and bile salt are as a rule similar to the concentrations of these substances in the gallbladder in cases of chronic cholecystitis. The cholesterol concentrations are, as a rule, considerably higher than those found in the bile from the normal gallbladder or in that from the chronically diseased gallbladder.

ABSENCE OF CHEMOTROPISM OF LYMPHOCYTES. HAROLD M. DIXON (by invitation) and MORTON MCCUTCHEON, University of Pennsylvania.

It is not understood how lymphocytes are accumulated in exudate, inflamed tissue and tubercles. Since one possible explanation is that these cells are attracted chemotropically, experiments were made to obtain evidence as to whether lymphocytes are capable of chemotropic reaction. Minute clumps (about 50 microns in diameter) of tubercle bacilli or of staphylococci were placed on a glass slide. A drop of blood from the finger was superimposed and allowed to spread between the slide and coverslip. With the microscope and a drawing ocular, the path of each lymphocyte and polymorphonuclear leukocyte was recorded on paper. For each white blood cell were determined (1) the net approach to the clump of bacteria and (2) the total path traversed in the same length of time. The ratio of these quantities is taken as the measure of chemotropism, +1 representing maximal positive, —1 maximal negative, chemotropism, and zero representing random movement or absence of chemotropism. When Staph. albus was used as the source of attraction, 60 polymorphonuclears showed strong positive chemotropism yielding a mean value of +0.65; in the same microscopic fields, the mean and the standard deviation for 45 lymphocytes were 0 ± 0.43. With tubercle bacilli, the figure for polymorphonuclears was +0.58; for lymphocytes, -0.10 ± 0.43. Hence, no evidence was obtained that lymphocytes are capable of chemotropism.

MORPHOLOGIC CHANGES IN THE CELLS FORMING EPIDERMOID CARCINOMA. JAMES E. DAVIS (introduced by F. W. HARTMAN), Wayne University.

The material available for this study was obtained from tissues taken from the cervix uteri for biopsy which illustrated various pathologic changes, including over 200 examples of carcinomatous changes of the epidermoid type. Various interesting phenomena have been observed in following the gradations of changes in areas where natural repair was occurring. In the early stage of this repair the most immature or basal cell types were quantitatively increased in the complete layer of cells forming the protecting epithelial stratum. With each succeeding repair the quantity of immature cells was increased, and where irritation and disarrangement of the natural relations of the cells prevailed, cell maturation showed still greater delay. In the different stages of the malignant changes the quantity of immature cells varied until the ultimate picture showed that all of the cells in the stratified epithelial layer produced a heterogeneous arrangement of the three different layers without any suggestion of the original differentiation of basal, middle and superficial strata.

THE SERUM PHOSPHATASE IN COMPLETELY DEPANCREATIZED DOGS. VICTOR SCHELLING (introduced by F. W. HARTMAN), Henry Ford Hospital.

The simultaneous decrease of inorganic phosphorus when sugar is removed from the blood has been interpreted as an intermediate compound of phosphorus

and hexose. In diabetic persons this fall is markedly delayed or even absent. As the formation of hexosephosphoric esters is presumably enzymatic, the serum phosphatase with an optimum p_H 8.6 (Bodansky) has been studied in completely depancreatized dogs. In normal animals kept on a standard diet (Cowgill) for a long period the serum phosphate in the postabsorptive state fluctuates from 1 to 2 units (Bodansky) at different times. After complete removal of the pancreas the serum phosphatase increases from 20 to 30 units. Insulin produced no greater changes than were observed as limits of fluctuation in normal animals. Intravenously injected dextrose had no significant effect on the serum phosphatase determined in blood removed at definite intervals. It is concluded that if any formation of hexosephosphoric esters takes place in the blood of completely depancreatized dogs under the influence of insulin, serum phosphatase with the optimum p_H 8.6 has no part in the reaction.

QUANTITATIVE STUDIES ON BLOOD COAGULATION. H. P. SMITH and (by invitation) E. D. WARNER and K. M. BRINKHOUS, University of Iowa.

Quantitative methods for the titration of prothrombin and antithrombin in plasma are outlined. In such titrations one must distinguish between the titer of the actual antithrombin and the titer of the total amount which can be developed in the plasma with the aid of heparin. It is shown that with peptone injected into the circulation of the dog the antithrombic activity of the plasma rises, but not the titer of the potential total. The prothrombin content is normal. An injection of india ink has much the same effect as an injection of peptone. Chloroform poisoning causes a profound fall in prothrombin and fibrinogen, together with a moderate fall in platelets and in antithrombin.

EFFECT OF TOTAL HEPATECTOMY ON THE BLOOD FATS. STEPHEN MADDOCK, Boston City Hospital.

In a series of dogs the blood fats were studied following total removal of the liver. It was found that a diminution in all of the constituents of the blood fat occurred in every instance. This change becomes appreciable at the twelfth hour and appears to be progressive throughout the remainder of the animal's life.

REFLECTION OF METEOROLOGICAL INFLUENCES IN THE SERUM REACTIONS (WASSERMANN, KAHN, ETC.). WILLIAM F. PETERSEN and (by invitation) E. T. HOVERSON and A. J. GOLDSTEIN, University of Illinois.

Pressor episodes occurring in man are predominantly meteorologically induced. With pressor episodes reorientation (autonomic, biochemical, etc.) of the entire organism follows, with somatic stimulation evident in many ways.

In view of the distinct environmental effect that is clinically apparent in syphilis, including neurosyphilis, it seemed possible that the serologic reactions might reflect alterations of the meteorological status, and studies were carried out to determine this point for both the Wassermann and the Kahn reactions.

When daily titrations were carried out in patients with dementia paralytica it was found that the titers of the Wassermann and Kahn reactions varied from day to day with a distinct increase occurring in the wake of pressor episodes (usually occurring with polar infalls). These effects may be cumulative. In this behavior the serum reactions merely resemble or reflect somatic changes which are closely interwoven with the meteorological environment.

ETIOLOGY OF AMYLOID DISEASE. HOBART A. REIMANN and (by invitation) CARL M. EKLUND, University of Minnesota.

Several theories have been advanced to account for the formation and deposition of amyloid substance: (1) that it is due to a general disturbance in the metabolism of protein; (2) that it represents an antigen-antibody union; (3) that it manifests disturbance or abnormality of the reticulo-endothelial system; (4)

that it is a feature of hyperglobulinemia, and (5) that it presents a combination of the conditions named in 3 and 4.

Theoretical considerations lead us to believe that a long-standing increase of normal or abnormal blood globulins is one of the chief factors since (*a*) hyperglobulinemia is present in many cases of long-standing infection, malignant tumor and myeloma, conditions frequently preceding amyloidosis, (*b*) there is an increase of blood globulins in the patients with amyloidosis, (*c*) amyloid disease may be produced experimentally by excessive feeding with protein or by repeated injections of vaccine, which are known to cause hyperglobulinemia.

Four rabbits were given about ninety intramuscular injections of sodium caseinate over a period of from eight to thirteen months. The amount of blood globulin was elevated after the first few injections and thereafter remained abnormally high (from two to four times over the normal level) until death. Early in the course the total amount of the blood protein was increased, but toward the end, when evidence of renal amyloidosis appeared, the level was subnormal. All the animals died, and at necropsy extensive amyloid disease was found. The greatest amount of amyloid substance was found in the kidneys; less was present in the liver and spleen.

VARIATIONS IN THE CATAPHORETIC MOBILITIES OF STREPTOCOCCI ISOLATED WEEKLY FOR ONE YEAR FROM THE NASOPHARYNGES OF TWO GROUPS OF WELL PERSONS, FROM RAW MILK AND FROM LABORATORY STRAINS. EDWARD C. ROSENOW and (by invitation) CAROL B. PRATT and CHARLES SHEARD, the Mayo Foundation.

The paper reports a parallel study of streptococci isolated weekly for one year from: (1) the nasopharynges of a group of nurses; (2) the nasopharynges of a group of laboratory workers; (3) raw milk; (4) strains maintained in the laboratory. The frequency distribution curves of cataphoretic mobility were so grouped as to be attributable to a cycle of changes in the electrophoretic characteristics of the bacteria in each group. Parallel changes in the streptococci from the four groups seemed to be correlated with certain seasonal epidemic respiratory infections.

TRANSMISSION OF EQUINE ENCEPHALOMYELITIS BY AEDES AEGYPTI. CARL TEN BROECK and (by invitation) MALCOLM H. MERRILL, the Rockefeller Institute for Medical Research.

Kelser's demonstration that the western strain of equine encephalomyelitis virus may be transmitted by Aedes aegypti gives us for the first time an insect-borne virus disease of animals that may be handled with ease in the laboratory. Our attempts to confirm his findings have brought out the fact that the mosquitoes must be fed a large amount of virus before they become infective. When they are fed small amounts, the virus can be demonstrated immediately, but it is lost in the course of a few days. We have also found that the western strain of encephalomyelitis virus will increase in the body of A. aegypti. It has been carried through a series of fifteen lots of mosquitoes by feeding normal mosquitoes on the crushed bodies of infected ones, and the virus has reached a dilution far beyond that which could infect if increase had not taken place. Another fact that has come out is that while A. aegypti will readily transmit the western strain of virus, it rarely is able to transmit the eastern strain. We have never succeeded in getting mosquitoes fed on suspensions of the brains of guinea-pigs infected with the eastern strain to transmit the virus by biting, while those fed on infected guinea-pigs occasionally are able to transmit it.

PROTEOLYTIC ENZYMES OF MONOCYTIC AND POLYMORPHONUCLEAR PLEURAL EXUDATES. CHARLES WEISS and (by invitation) E. J. CZARNETZKY, Mount Zion Hospital, San Francisco.

This article will be published in full in a subsequent issue of the ARCHIVES.

PHILADELPHIA PATHOLOGICAL SOCIETY

Regular Meeting, Feb. 14, 1935

MORTON MCCUTCHEON, *President, in the Chair*

PARAGANGLIOMA (?) OF THE ADRENAL MEDULLA. JOHN EGOVILLE.

A markedly emaciated white Englishman of 59 years had complained of increasing weakness for a year. There were several episodes of vertigo with falls and injury. One week before admission to the hospital he had visual hallucinations. Four years before death his blood pressure was 150 systolic and 60 diastolic, and about six hours before death it was 140 systolic and 120 diastolic. Death was attributed to cardiac failure.

Necropsy revealed severe congestion of all the organs with areas of degeneration in the right diencephalon and both mamillary bodies. The heart weighed 210 Gm. and was soft and dull brown. The kidneys were involved by benign nephrosclerosis. The left adrenal gland contained a circumscribed, spherical, gray-brown medullary tumor, 1.5 cm. in diameter, surrounded by a thin layer of cortex; the cut surface was studded with deep red pinpoint dots. The opposite adrenal gland was normal. Secondary tumors were not seen.

In histologic sections the tumors was unencapsulated and consisted mainly of large polyhedral cells having a palely staining cytoplasm and vesicular nuclei. Large numbers of the cells contained dustlike pigment, which gave the specific green chromaffin stain with Giemsa's method. Some areas showed a dense admixture of small round, oval or spindle-shaped cells, the last having long polar processes which extended along the alveolar septums. Throughout the mass extensive hemorrhage and necrosis had occurred with deposits of hemosiderin, accompanied by lymphocyte and plasmocyte infiltration. Large cavernous spaces filled with a clear pink staining fluid (lymph) formed the center. The stroma was finely fibrillar.

The relation of the tumor to the hypertension is on purely speculative ground. The heart was small and not of the hypertensive type; it is suggested that the hypertension may have been paroxysmal, possibly accounting for the episodes of vertigo.

NEUROBLASTOMA OF THE ADRENAL GLAND WITH HEPATIC METASTASIS SIMULATING VON GIERKE'S DISEASE. EDWARD S. THORPE JR.

An infant of 3 months was admitted to the pediatric service of the Philadelphia General Hospital on Sept. 9, 1934, presenting chiefly enlargement of the abdomen and constipation. Its birth had been normal, and its development up to a few weeks before admission to the hospital had been uneventful. The only physical findings of note were a moderate secondary anemia, a leukocytosis of 21,000, of which 87 per cent were lymphocytes, and enlargement of the liver. The liver was so large that the mass filled both flanks and extended below the symphysis pubis. In the belief that the diagnosis was glycogenic hepatomegalia (von Gierke) the condition was studied along the lines indicated by van Creveld. The child showed a constant hypoglycemia which was affected very little by the injection of epinephrine, a constant ketonuria which showed a paradoxical reaction following the injection of epinephrine, a slight increase in the blood glycogen and a negative reaction to the Takata-Ara test for hepatic function. Twelve days after admission of the baby to the hospital, the temperature rose abruptly and it died, apparently with edema of the lungs.

Significant observations at necropsy were: a tumor, 2.3 by 3 by 3.5 cm., in the left adrenal medulla; a tumor 2 mm. in diameter in the right adrenal medulla, and almost complete replacement of hepatic tissue by a secondary tumor, the liver weighing 1,230 Gm. The primary adrenal tumors were well circumscribed and sharply demarcated from the overlying cortex, the tumor tissue being mottled

dark red to red-gray and trabeculated with fine strands of fibrous tissue. Histologically the tumor was composed almost exclusively of circlets of small hyperchromatic lymphocyte-like cells ("rosettes") with a fine pink-staining central tuft; the intervening stroma was hemorrhagic, and large areas of necrosis were prominent. The behavior of the tumor, i. e., metastasizing exclusively to the liver, characterized it as the so-called Pepper type of neuroblastoma.

NEUROBLASTOMA OF THE ADRENAL MEDULLA (HUTCHISON'S TYPE). D. R. COMAN.

A colored girl, aged 2 years, was admitted to the hospital, Sept. 22, 1934, with the history that three weeks previously she had been taken ill with malaise, fever, vomiting, a loss of weight and constipation. Her temperature on admission was 101.2 F. Physical examination revealed a rough systolic murmur, a prominent abdomen, an umbilical hernia, a palpable liver, a large abdominal mass and 2,600,000 red blood cells per cubic millimeter with 38 per cent hemoglobin. The child failed rather rapidly and died on October 5.

The clinical diagnosis was retroperitoneal tumor.

At autopsy the abdominal cavity contained about 500 cc. of bloody fluid, and the retroperitoneum showed scattered punctate hemorrhages. The entire central portion of the abdominal cavity was occupied by a large mass reaching to the diaphragm in the left upper quadrant, coming into close relationship with the under surface of the liver and extending downward to the pelvic brim. The omentum was found riding over the mass, extending from the greater gastric curvature above to the transverse colon lying just below the most prominent portion of the tumor. The kidneys were found lying on either side of the neoplasm, somewhat distorted by its pressure, but not incorporated in it. No adrenal gland was found on the left side; that on the right side was in its usual position between the upper pole of the kidney and the under surface of the liver. The surface of the tumor was lobular and smooth. It was cystic to palpation in some areas and solid in others. It was gray with red splotches scattered here and there, and on section displayed areas of gray, soft bloody tissue, with other cystically dilated portions filled with bloody fluid. Other areas were semifluctuant, purplish and necrotic.

There was no gross evidence of metastatic involvement of the other abdominal, pelvic or thoracic viscera. The skeleton, however, showed widespread metastases. These were found in the sternum, ribs, femurs and skull and appeared as slightly elevated dark red patches of variable size and irregular shape. When the orbital plates were removed, masses of soft, dark red, friable tissue were found in the retro-orbital spaces. The amount of this foreign tissue was not sufficient to cause exophthalmos.

The gross anatomic diagnosis was neuroblastoma of the Hutchison type arising from the left adrenal medulla.

Histologic preparations revealed, in addition to the grossly visible metastases, involvement of the liver, kidneys and spleen. The tumor was seen to consist of masses of small round cells appearing not unlike lymphocytes. There were large areas of necrosis and hemorrhage. The cell type was uniform throughout, and the stroma was of variable quantity.

Examination under high power showed the cells to be neuroblasts, rather larger than lymphocytes, with relatively more cytoplasm and large vesicular deeply basophilic nuclei. They were arranged in clumps and strands. No typical "rosette" arrangement could be found.

This tumor conforms to the Hutchison type of neuroblastoma of the adrenal medulla.

GENERALIZED TUBERCULOSIS IN THE NEW-BORN. HARRY M. STEEN.

A woman, aged 27 years, was admitted to the obstetric ward of the Presbyterian Hospital in labor on Nov. 16, 1933, and delivered of a living full-term female child weighing 7 pounds and 9 ounces (3,430 Gm.). The mother gave a history of a

severe respiratory infection occurring during the third month of pregnancy, at which time she was confined to bed for two weeks. The mother was seen for the first time in the prenatal clinic on July 17; she had the physical signs and roentgen evidence of a pleural effusion of the left side. The diagnosis on admission was questionable pulmonary tuberculosis, a diagnosis which was subsequently strengthened by roentgen studies and physical findings during her stay in the hospital. However, neither the roentgenologists nor the clinicians would commit themselves to a diagnosis of active tuberculosis. Two examinations of the sputum and one of the gastric residue disclosed no acid-fast bacilli.

The baby lived twenty-five days. The clinical course was as follows: On the second day, the baby had a temperature of 100.4 F. From the second to the fifth day, the fever ranged between 100.4 and 102.4. The baby was transferred from the obstetric ward to the pediatric service of Dr. Charles A. Fife. On the fifth day, there was slight vaginal bleeding. The baby was thought to have hemorrhagic disease of the new-born, and 20 cc. of the mother's blood was injected intramuscularly into the buttocks. The spleen was enlarged and extended out from the costal margin 1 cm. On the seventh day, the baby was placed at the mother's breast for the first time and received five feedings. On the eighth day, the vaginal bleeding stopped, and the temperature was 103 F. The baby was removed from the breast and placed on a formula. On the tenth day, the baby was slightly dehydrated; the spleen was larger, and the x-ray films showed a general fuzziness of the chest, especially about the shadows of the roots of the lungs. There was a decreased resonance of the apex of the right lung. On the eleventh day, the baby was more dehydrated; the spleen was larger; the liver was enlarged, and the abdomen was distended but soft. The Mantoux test was positive. On the eighteenth day, the febrile course continued, and there was a positive reaction to tuberculin (0.001 mg.). The liver was palpable almost to the level of the umbilicus. On the twenty-first day, the gastric residue and feces contained acid-fast organisms in large numbers. On the twenty-fourth day, resonance was impaired generally over the chest, and fine crepitant râles were heard all over the chest. Death occurred on the twenty-fifth day of life.

November 16, the Wassermann and Kahn tests of the cord were negative. November 20, the bleeding time was four minutes and thirty seconds; the coagulation time, three minutes. November 25, the hemoglobin was 110 per cent. The red blood cell count was 5,120,000; the white cell count, 11,650. The polymorphonuclears were 52; the small lymphocytes, 46, and the undifferentiated cells, 2 per cent. December 4, the hemoglobin was 106 per cent. The red blood cell count was 4,320,000; the white blood cell count, 12,150. The polymorphonuclears were 88; the small lymphocytes, 11, and the moonocytes, 1 per cent. December 6, guinea-pigs inoculated with gastric residue showed tuberculosis. December 9, guinea-pigs inoculated with spinal fluid and blood cultures showed tuberculosis. Guinea-pigs given injections of the mother's milk disclosed no evidence of tuberculosis.

At necropsy general miliary tuberculosis was found. The brain and long bones were not involved.

Microscopically, the tubercles in the involved viscera appeared to be of the same age and were exudative. Acid-fast organisms were demonstrated in sections of the lungs, liver, spleen, kidneys and adrenals. Numerous sections through the umbilical vein showed no tuberculous involvement. The placenta was discarded at the time of delivery.

Recognizing that the five breast feedings and the injection of the mother's blood introduce a reasonable doubt, I still feel that the absence of clinical or roentgenologic evidence of open tuberculosis in the mother and the negative findings in guinea-pigs given injections of the mother's milk confirm the belief that this case is an example of transplacental infection.

PRIMARY (?) TUBERCULOUS PERICARDITIS. A. I. RUBENSTONE.

A Russian Jew, aged 64, was admitted to Mount Sinai Hospital, Feb. 6, 1933, complaining of precordial and epigastric pain. For about six years he had had hypertension, and attacks of precordial pain had often followed physical effort. These symptoms developed following right hemiplegia, from which recovery had been gradual but complete. He was fairly well nourished, dyspneic and moderately cyanotic at rest; the blood pressure was 202 systolic and 95 diastolic. The temperature was 99 F.; the pulse rate was 104 and the respiratory rate 24. The supracardiac area was slightly broadened; the heart was moderately enlarged in all diameters; the sounds were rhythmic and of poor quality, with A2 markedly accentuated. There was evidence of diffuse fibrosis of the lungs, with râles at both bases; there were tenderness and rigidity of the right hypochondrium, and the liver was definitely palpable; there was slight pretibial edema.

The presumptive diagnosis was advanced arteriosclerosis, with coronary involvement, hypertension and decompensation, and early bronchopneumonia. Cytologic, chemical and serologic examination of the blood gave negative results. The urine was normal. Roentgen examination of the chest confirmed the physical evidence of moderate cardiac enlargement and also revealed congestion of the basal portions of the lungs. The electrocardiogram revealed evidence of myocardial involvement but not of acute coronary occlusion.

During the month's hospitalization, the temperature varied from 99 to 101 F. Venesection with various medicinal measures resulted in more comfort for the patient, with a drop in the systolic blood pressure to 160, but the anginal attacks became more frequent and severe and were accompanied by increased cyanosis and dyspnea. The pulmonary involvement steadily increased, accompanied by a gradual weakening of the cardiovascular mechanism. The patient died on March 6.

At autopsy the anterior surface of the pericardium was normal. The parietal pericardium was greatly thickened. About 100 cc. of bloody fluid was removed from the pericardial sac, revealing a hemorrhagic pericarditis of the "bread and butter" variety involving the entire surface of the heart and ending abruptly at the point of reflection of the pericardium.

The mitral and aortic valves were normal except for moderate atheromatosis. The left ventricular wall was 1.8 cm. thick. The right coronary vessels and the left circumflex branch showed some atheromatous change but no encroachment on their lumens. In addition, the left descending branch in one region showed definite constriction of the lumen. Histologically, the epicardial layer was thickened by multiple tubercles of the productive variety. The tuberculous process stopped abruptly at the myocardial border except in a few areas in which a slight infiltration of round cells in the outer muscle layers was noted. There was extensive fibrosis of the lungs with diffuse pneumonitis, terminal infarction and atelectasis. Careful search for other tuberculous foci revealed only a few prominent fibrotic mediastinal lymph nodes some distance from the pericardium. Histologic study revealed a questionable reactivation of very old tuberculous lymphadenitis.

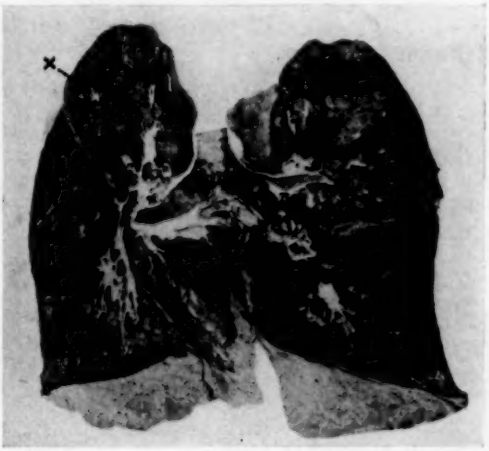
CLINICAL AND PATHOLOGIC NOTES ON A CASE OF HEALED PULMONARY TUBERCULOSIS. F. MAURICE MCPHEDRAN and ESMOND R. LONG.

Perfectly healed tuberculosis of the adult type and of unknown clinical course is frequently seen at necropsy. Tuberculosis of known course is seldom seen. It seems worth while, therefore, to put a case, recently observed, on record.

A colored man, 34 years old, entered the clinic of the Henry Phipps Institute in August 1928 with a history of cough of five months' duration. He was well nourished, weighing 187 pounds (84.8 Kg.). He had lost 18 pounds (8.2 Kg.) some months previously, but shortly regained the loss. Abnormal physical signs in the chest were inconstant. Roentgen examination showed groups of very light spots in the posterior fourth and fifth interspaces on both sides. Tubercle bacilli in small numbers were found in the sputum in two of three examinations in August.

In 1929, 1930 and 1932 he had acute respiratory symptoms, with roentgen evidence of bilateral basal nontuberculous bronchopneumonia and associated basal râles. The groups of light spots visible in the upper lobes in 1928 were no longer apparent in the x-ray films, nor were physical signs referable to this part of the chest. Repeated examinations of the sputum between August 1928 and June 1934 disclosed no tubercle bacilli. During these years headaches, polyuria and visual defects developed. Roentgen examination of the skull disclosed an enlargement of the sella turcica. An operation for tumor of the hypophysis performed on June 28, 1934, was unsuccessful, and the patient died in a state of shock a few hours afterward.

In addition to the hypophyseal tumor—an acidophilic cell adenoma—the autopsy disclosed significant changes only in the lungs. In the posterior parts were edema and early inflammatory change. The lungs were fixed by filling them through the trachea with Kaiserling's solution 1, and after thorough hardening they were cut into slices 1 cm. thick. In the outer portion of the middle third of the right upper lobe was a scar which had an anthracotic margin with radiating extensions out-



Isolated healed tuberculous lesion of adult type in the upper lobe of the left lung, believed to have been the source of the tubercle bacilli in the sputum five years previous to the death of the patient (death from tumor of the brain).

ward. The scar measured 7 by 9 by about 7 mm. It surrounded a softer, white, partly fibrous mass, measuring 6 by 3 by 4 mm. No changes, fibrous or otherwise, were found in the apex, but there was a calcified tubercle, measuring 2 by 3 by 2 mm., just internal to the pleura directly posterior to the scar described.

No other significant gross changes were found in this lobe. In the right lower lobe was a calcified tubercle measuring 8 by 4 by 8 mm. In the regional pulmonary lymph nodes was a collection of hard calcified masses, totaling 1 cm. in diameter, and a calcified mass 8 mm. in diameter was present in a lymph node below the right main bronchus close to the bifurcation. Congestion, edema, thrombotic emboli, early hemorrhagic infarcts and early inflammatory consolidation were seen elsewhere in the lungs.

The diagnosis was healed tuberculosis of the childhood type in the right lower lobe and healed ulcerative tuberculosis of the adult type in the left upper lobe. Microscopic sections of the latter lobe disclosed a dense fibrous wall and a necrotic center, infiltrated at the margins with lymphocytes and fibroblasts. The usual

histologic criteria of activity were absent. Tubercle bacilli could not be found. (Obviously, because of the fixation necessary in finding the lesion, experiments in inoculation were ruled out.) The bronchus leading to the region of the scar was somewhat contracted by fibrous tissue, and its lumen was almost filled by accumulated, in part degenerated, epithelial cells. The artery to the part was much thickened, with a greatly reduced lumen. In the regional lymph nodes, in addition to anthracosis, there was slight fibrous scarring.

It was believed that the scar in the right upper lobe represented the ulcerative lesion responsible for the sputum containing tubercle bacilli six years before. It is altogether probable that this lesion in turn was secondary to the subpleural lesion described, and that the latter was the first lesion of reinfection.

CHANGES IN THE PITUITARY FOLLOWING THYROIDECTOMY IN RATS. ISOLDE T. ZECKWER, LEWIS W. DAVISON, THOMAS B. KELLER and CLARENCE S. LIVINGOOD II.

A paper on this subject was read before the American Society for Experimental Pathology, the abstract of which appears on page 159.

DYSONTOGENETIC ORIGIN OF BASAL CELL CARCINOMA. JOSEPH MCFARLAND, E. F. CICCONE and JOSEPH GELEHRTER.

We endeavored to confirm or refute Glasunow's theory that the basal cell carcinoma develops from imperfections in the closure of the embryonal facial fissures. A consideration of the theories as to the nature and origin of the basal cell tumors and of the diversified histologic structure suggests that the tumors arise from something more primitive than the perfected epiderm. Attention is also called to the fact that no basal cell tumor has ever been produced experimentally.

Glasunow collected 254 cases of basal cell carcinoma, 112 of which after histologic confirmation had their exact localization on a diagram of the face. Their distribution conformed almost perfectly with the location of the embryonal facial fissures.

We culled 50 cases from the material in the Philadelphia General Hospital and 65 cases from that in the American Oncologic Hospital and similarly indicated the localizations on a facial diagram. With the exception of a few scattered cases the carcinomas tended to fall into positions in fair correspondence with those of the embryonal facial fissures.

As there is lack of agreement as to the histology of basal cell tumors, Ewing's criterion was adopted, viz.: "The normal transformation into squamous cells entirely fails."

In order to support the evidence by comparison, two other types of dysontogenetic lesions, similarly plotted, were studied. These were the so-called sequestration dermoids and the facial mixed tumors. For the former, Forgue's diagram was borrowed. With the exception of one or two lesions aberrant on the forehead, the correspondence was complete. The aberrant lesions may have been sebaceous cysts mistaken for dermoids.

One of us (Dr. McFarland) reviewed the entire literature of mixed tumors of the face, lip, nose and supra-orbital regions, noting their reported localizations, and plotted them on a similar diagram. Again, with very few exceptions, which may relate to errors in diagnosis, the tumors conform to the distribution of the embryonal facial fissures.

So the distributions of the basal cell tumors, the sequestration dermoid cysts and the mixed tumors are the same, and the number of aberrant cysts and tumors is not of sufficient importance to discredit the theory.

Another comparison to act as a control was made by similarly plotting 100 cases of squamous cell lesions of the face (50 from each institution). The result is extraordinarily different, and the distributions have no relation to the fissures.

Conclusion.—The results obtained are in complete accord with those of Glasunow and support his theory that basal cell carcinoma is of dysontogenetic origin and arises from ectodermal cells sequestered in the concrescence of the embryonal facial fissures.

Book Reviews

On the Occurrence of Lymphogranulomatosis (Sternberg) in Sweden 1915-1931 and Some Considerations as to Its Relation to Tuberculosis. By Martin Uddströmer. Acta Tuberculosea Scandinavica, Supplementum I. Copenhagen: Levin & Munksgaard, 1934.

Cases of lymphogranulomatosis in Sweden from 1915 to 1931 numbered five hundred and forty-eight. The average rate of incidence is two hundred and eighty-eight times less than that of tuberculosis for the years from 1915 to 1930. The absolute geographic distribution of lymphogranulomatosis coincides with the more densely populated areas. Regional differences are found, but are evenly distributed between the rural and urban populations. Several facts argue against the contagiousness of lymphogranulomatosis; social and hygienic conditions do not seem to be of importance.

Males preponderate over females in number, and this is accentuated in childhood, speaking against occupational factors in the relative sexual morbidity. Ovarian hormones as protective influences for the female do not seem to play a part.

Age is held to be a predisposing factor, the disease being exceedingly infrequent during the first two years of life, not common up to the age of puberty and showing a marked increase immediately thereafter. The peak of the curve for age does not form a maximum, except for females. The disease occurs frequently among all age groups, in males with increased incidence to form a maximum at about 60 years. The relative age at incidence in lymphogranulomatosis conforms only in part with that in tuberculosis.

As to duration, 60 per cent of the patients died in less than two years, and 87 per cent in less than four years. The data for those aged 60 years or more show a shorter duration.

"Neither in geographical distribution nor in family histories, clinical and post-mortem cases do we find a coincidence of L.G. with tuberculosis that can lend support to any connection between the two diseases beyond the fact that L.G. favors the setting in of miliary tuberculosis, where in all post-mortem cases older tuberculous changes have been present." Tuberculin reactions are more frequently negative than is generally stated.

A percutaneous portal of entry is not favored, but the material seems to lend support to a possible gastro-intestinal portal in some cases, this tract being involved in at least one third of the cases. Splenomegaly is found in two thirds of the cases. The peritoneum is rarely involved. The abdominal lymph nodes, especially the retroperitoneal nodes are preponderantly affected. The lungs are affected in about one third of the cases, and usually the nodular form of the disease is encountered. It is difficult or even impossible at times to differentiate either clinically or at postmortem examination between lymphogranulomatosis and tuberculosis. The hemorrhagic diathesis is not often encountered. Leukocytosis occurs in many cases, being somewhat accentuated in advanced stages. Neutrophils are likewise increased, and the great majority of patients show normal or decreased eosinophils. Anemia is generally mild, but is present in about one half of the advanced cases and in about one third of the early cases. The sedimentation reaction always shows raised values, which are often marked and may be considered of prognostic importance. Normal values must not be taken as a sure sign of prolonged duration. High rates occur more frequently in lymphogranulomatosis than in tuberculosis.

Records of several rare cases seen clinically and post mortem are given. An extensive bibliography is included.

The reviewer wishes to call attention to this monograph as being the most detailed and exhaustive survey of a single phase of lymphogranulomatosis yet published. The most striking observation resulting from this work would appear to be that there is no coincidence between lymphogranulomatosis and tuberculosis other than the occurrence of terminal miliary tuberculosis in association with some cases of lymphogranulomatosis.

Experimental Bacteriology in Its Applications to the Diagnosis, Epidemiology, and Immunology of Infectious Diseases. Volumes I and II. By Dr. W. Kolle, Director of the Institute for Experimental Therapy and of the Chemicotherapeutical Research Institute "Georg Speyer-Haus," Hon. Professor at the University of Frankfort, and Dr. H. Hetsch, Professor at the Institute for Experimental Therapy, Frankfort. Translated from the seventh, completely revised German edition by Dagney Erikson, the English version, incorporating further revision, edited by John Eyre, F.R.S. (Edin.), F.Z.S., M.D., M.S., D.P.H., Director of the Bacteriological Department, Guy's Hospital, Professor of Bacteriology, University of London. Price, $16. Pp. volume I, 592; volume II, 613, with 118 plates and 200 text figures. New York: The Macmillan Company, 1935.

The first edition of this book appeared in 1906. Through the years the successive editions have been accepted as giving a true and authoritative presentation of the science and art of bacteriology in theory and practice according to the traditions of the Koch school. Translations have been made into several European languages, the most recent being this into English from the seventh and last German edition. The first volume begins with general bacteriology and immunology, after which come twenty-seven chapters on infectious diseases, nearly all of bacterial origin. The second volume deals with tuberculosis, leprosy, spirochetal and protozoan diseases, diseases due to filtrable viruses, infectious diseases of unknown causes (in which are included beriberi, pellagra and scurvy) and diseases due to fungi and yeasts. Because the two volumes constitute an organic whole it is unfortunate that each is treated separately in the matter of pagination and index, especially as there are no indications in the titles as to what each volume contains. There should have been consecutive pagination and one common index. The translation impresses one favorably. The illustrations are admirable, particularly the colored plates. The old names of bacteria are used consistently and exclusively; the indexes do not contain any newer names, such as Corynebacterium, Mycobacterium, Neisseria, Eberthella, Escherichia and Clostridium. Bacterial dissociation or pleomorphism is not discussed; the old monomorphic conception holds full sway. In several other respects also the book has failed to keep step with the advances, e. g., in its discussion of bacterial food poisoning, of typhus fever, of brucella infection, of streptococci in relation to epidemic sore throat and to specific toxin production in erysipelas. The main value of the book to the student of microbiology and infectious diseases lies in its portrayal of the wonderful developments in those fields in Germany under the influence of the great pioneers of the Koch era.

The Kidney in Health and Disease. In Contributions by Eminent Authorities. Edited by Hilding Berglund, M.D., Stockholm, Sweden, formerly Chief of the Department of Medicine at the University of Minnesota, and Grace Medes, Ph.D., Research Biochemist in the Lankenau Hospital Research Institute, Philadelphia, with the Collaboration of G. Carl Huber, M.D.; Warfield T. Longcope, M.D., and Alfred N. Richards, Ph.D., M.D. Cloth. Price, $10. Pp. 754, with 163 engravings. Philadelphia: Lea & Febiger, 1935.

This book contains the revised and amplified papers contributed to the symposium on the kidney at the University of Minnesota in the summer of 1930. This symposium was organized by Hilding Berglund, then professor of medicine in the university. Since his return to Sweden the editorial work has been completed by Grace Medes and her collaborators. The book contains forty-four articles, and

there are forty-one contributors. The contents are divided into six parts: the anatomy and physiology of the kidney, the clinical aspects of renal functions, Bright's disease and various other pathologic renal conditions, albuminuria and edema, ocular changes in Bright's disease and the clinical aspects of Bright's disease. To attempt to review the individual articles is out of the question. The authors represent well the active investigators and students of the kidney in this country; European visitors contribute several articles. The editorial work has been done carefully. There are good indexes. The book is an example of a high standard of book-making. It gives a comprehensive review of present knowledge of the kidney in health and disease which will prove helpful to all who are interested in that field.

Books Received

A Bibliography of the Poem Syphilis Sive Morbus Gallicus by Girolamo Fracastro of Verona. Leona Baumgartner and John F. Fulton. Price, $5. Pp. 157, with 10 illustrations. New Haven, Conn.: Yale University Press, 1935.

The International Cancer Research Foundation; Report of Activities During 1934. Pp. 79.

Memorial Meeting in Honor of William Henry Welch, Held at the University Club, Tuesday, May 22, 1934. Pp. 44, with 1 illustration. Baltimore: The University Club, 1935.

Key-Catalogue of Parasites Reported for Carnivora (Cats, Dogs, Bears, Etc.) with Their Possible Public Health Importance. C. W. Stiles and Clara Edith Baker. National Institute Health Bulletin No. 163 (Continuation of Hygienic Laboratory Bulletin Series), December 1934. Price, 20 cents. Pp. 913-1223 (pagination continuous with that of Bulletin 159). For sale by Superintendent of Documents, Washington, D. C. Washington, D. C.: United States Government Printing Office, 1935.

Collected Reprints from the Laboratories of the Mount Sinai Hospital, New York, Louis Gross, M.D., Director, 1934.

Report of the Laboratory and Museum of Comparative Pathology of the Zoological Society of Philadelphia in Conjunction with the Sixty-Third Annual Report of the Society. Herbert Fox, M.D., Pathologist. Pp. 32. 1935.

Experimental Studies on Cancer. Carl Voegtlin and Others. National Institute of Health Bulletin No. 164, January 1935. Price, 10 cents. Pp. 58, with 4 plates. For sale by Superintendent of Documents, Washington, D. C. Washington, D. C.: United States Government Printing Office, 1935.

Contribution à l'étude de la variabilité du virus tuberculeux. P. Denys. Pp. 90, with 6 figures. Louvain: Imprimerie Saint-Alphonse, 1935.